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# THE VALIDITY OF RAPID DETERMINATIONS OF THE OSMOTIC PRESSURE OF PROTEIN SOLUTIONS<sup>1</sup>

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The methods for measuring osmotic pressure which are at present acceptable to the more critical investigators (1, 2) often require weeks or months to carry out. Membranes must be selected with so slight a degree of permeability that there can be no question of the escape of solute molecules into the outer solution. Many days may be required to attain equilibrium, and it is considered good practice to maintain the final pressure over a long period in order to make certain that a true equilibrium has been established. When protein solutions are used it is customary to preserve them against putrefactive or other changes by conducting the experiment at 0° C. However, not all protein solutions remain stable under these conditions; and, in fact, no method has as yet been found which will preserve blood serum or other biological fluids in an osmotically stable condition for a sufficient period to allow the determination of the original osmotic pressure by the standard methods.

The use of relatively permeable membranes in attempts to measure the osmotic pressure of serum proteins by the various rapid methods now in common use for clinical investigations is a questionable practice, unless it can be demonstrated conclusively that these membranes are strictly semipermeable. In the absence of a standard preparation of serum of known osmotic pressure it will be necessary, alternatively, to demonstrate the accuracy of such rapid methods by tests carried out with some other protein solution, the osmotic pressure of which has been accurately determined by an orthodox method. It must further be shown that serum proteins remain in an osmotically stable condition during the time required for the determination. The data to be presented indicate that the rapid method in use in this laboratory (3, 4, 5, 6) for studies on bio-

logical fluids does indeed measure up to these criteria of reliability.

## PROCEDURES

The determinations of the osmotic pressure were carried out as described previously, except that the osmometers, supported on convenient racks, were submerged below the surface of water in a bath, the temperature of which could be maintained constant within  $\pm 0.05^\circ$  C. or better. The temperature of 0° C. was maintained by the addition of cracked ice, thoroughly stirred with air and prevented from blanketing the osmometers by being held in the center of the bath behind a barrier of fine wire screen. Other temperatures below and above that of the room were obtained by a combination of cooling coils and heater or by the heater alone, in the usual manner. The necessity of maintaining a constant temperature is obvious when one considers that a change in temperature of 0.1° C. or less will produce an appreciable change in volume of the solution, with a corresponding movement of the meniscus in the capillary tube of the osmometer.

It has been found that blood serum will usually undergo irreversible changes at 0° C. Osmotic equilibrium cannot be established, the pressure continues to fall, and a definite precipitation of protein occurs. At temperatures above 25° C., on the other hand, serum, when collected in the usual way without special aseptic precautions, will occasionally, but by no means invariably, develop putrefactive changes within the time necessary for the attainment of equilibrium. In the studies on the relation of the osmotic pressure to temperature, therefore, the pressure was determined first at the lowest temperature (which was never below 10° C.). A very few hours at each of the higher temperatures would then suffice for the attainment of a new equilibrium, without danger of putrefactive changes. In a few instances the temperature was again lowered to the original level and the pressure redetermined. The fact that these two determinations at the original low temperature gave values which checked well within the limits of error of the method was considered as positive evidence that the serum had not undergone irreversible changes during the course of the experiment.

Hemoglobin solutions may be kept almost indefinitely at 0° C. as Adair (1) found; but putrefaction occurs readily at higher temperatures. Errors due to such changes may be avoided if one conscientiously adheres to the rule that no pressure value shall be considered a true equilibrium pressure unless the meniscus of fluid in the osmometer can be kept at an absolutely constant level

<sup>1</sup> This study has been aided by a Fluid Research Fund granted by the Rockefeller Foundation.



(at constant temperature) for a period of at least two hours. A continuous fall of the pressure, no matter how gradual, which persists long after the time usually required to attain equilibrium, is prophetic of the obvious putrefaction or precipitation of protein which is to follow.

Membranes of several specific permeability ranges were prepared by varying the concentration of alcohol in which the dry collodion sacs were allowed to swell. For this purpose alcohol-water solutions were prepared as follows: 91 cc. of absolute alcohol (density,  $d_{20}^{20}=0.7816$ ) plus 9 cc. water, giving a solution of density 0.8124 and weight per cent 88.79 and providing membranes in the specific permeability range (3) of 1 to  $3 \times 10^{-8}$ ; 93 cc. alcohol plus 7 cc. water, density 0.8062, weight per cent 91.14, permeability range 6 to  $12 \times 10^{-8}$ ; 95 cc. alcohol plus 5 cc. water, density 0.7997, weight per cent 93.50, permeability 20 to  $57 \times 10^{-8}$ ; and 97 cc. alcohol plus 3 cc. water, density 0.7924, weight per cent 96.10, giving the range of permeability of 200 to  $300 \times 10^{-8}$ . Membranes of the latter group are usually distinctly permeable to serum proteins, but in one instance an equilibrium was attained, without leakage of protein. The "95-alcohol" grade of membranes may be considered the standard, as they are the most practical to use for ordinary purposes. They have been employed throughout the present investigation, except for certain of the experiments reported in Table II.

In several instances, during the past few years, an ultrafiltrate of serum has been prepared for use as outer solution. Serum was ultrafiltered through the standard membranes, as defined above, at a pressure of 300 to 500 mm. Hg. During the course of 2 or 3 hours, 1 cc. or more of ultrafiltrate was obtained. A fresh membrane was always employed for the measurement of osmotic pressure. While the use of the ultrafiltrate may give initial pressure readings which are much closer to the equilibrium values (within 30 mm. of water, or thereabouts) the time required to attain equilibrium was not appreciably shortened in any of our experiments. The same equilibrium was obtained as when 0.9 per cent sodium chloride or Ringer-Locke's solution was used as the outer solution. Consequently there seems to be no advantage to be gained from the use of ultrafiltrates.

Hemoglobin solutions were prepared from oxalated human blood of normal individuals. The blood was centrifuged and the serum removed. The cells were washed four times in the centrifuge with an equal volume of Ringer-Locke's solution of the following composition: NaCl, 0.9 gram per 100 cc.; KCl, 0.042;  $\text{CaCl}_2$ , 0.024;  $\text{NaHCO}_3$ , 0.02. No glucose was added. The pH of the solution by the method of Cullen (7) was 8.00. After washing, the corpuscles were laked by adding an equal volume of water and 3 mgm. of saponin per 1 cc. of cells. One-half volume of triple strength Ringer-Locke's solution was then added to make up the normal salt concentration. In each experiment two or three dilutions were prepared from the original concentration (which contained from 12 to 14 grams of hemoglobin

per 100 cc. of solution). The concentration of hemoglobin in each solution was determined by the refractometric method of Stoddard and Adair (8). The readings of the Abbe refractometer were sharp for hemoglobin concentrations below 10 per cent, but much less accurate for the more concentrated solutions. The observed values of the lower concentrations were related to each other as their dilution factors, within  $\pm 0.2$  per cent. In one experiment the concentrations of four solutions were determined also by the oxygen capacity method of Van Slyke. The values checked those of the refractometer within  $\pm 0.2$  per cent hemoglobin.

#### DATA

Table I presents data relating to the effect of temperature on the osmotic pressure of blood serum and of hemoglobin solutions. The findings indicate that serum remains osmotically stable to variations in temperature from  $10^\circ \text{C.}$  to  $37^\circ \text{C.}$  during a sufficient period of time to allow the determinations to be carried out. The changes in osmotic pressure which occur under these conditions are reversible and are approximately of the magnitude to be expected on the assumption that serum behaves, osmotically, as an ideal solution. The possibility that cooling the serum below body temperature produces significant chemical or colloidal changes in the proteins is apparently ruled out. Consideration of the data on hemoglobin leads to similar conclusions. The determinations were carried out in duplicate. The "observed osmotic pressure change" was taken as the mean of the changes in pressure observed in the individual osmometers. The "theoretical change" was calculated from the mean of the pressure readings at the lowest temperature, on the assumption that pressure is proportional to absolute temperature. The ratio of the pressure change observed to that calculated in each case is given in the table. The variability of these ratios in individual experiments is to be attributed, in part at least, to variations in the capillarity of the solution in the osmometer tubes during the course of the experiment. The error of determining the average capillarity correction by observing the heights at which the rising and falling solution comes to rest in the osmometer tube at the end of the experiment should not appreciably affect the accuracy of the calculations, provided the actual forces of capillarity remained constant in each tube throughout the experiment (which often lasted 24 hours or longer). Various lines of evidence, such as

TABLE I

*The relation of the increase in osmotic pressure of human blood serum and hemoglobin solutions to changes in temperature, over different ranges, as compared with the changes which would be expected to occur in ideal solutions*

Sample	Subject	Ratios: $\frac{\text{observed osmotic pressure change}}{\text{theoretical change}} = \frac{\Delta\pi/\pi_1}{\Delta T/T}$							Average ratio for all temperature ranges	
		0°-20°	0°-25°	10°-20°	10°-30°	10°-37°	15°-35°	20°-37°	Serum	Hemoglobin
Serum	Bi			.537	.910	.672		.775	.724	
"	Ca			1.042	1.025	1.020		1.026	1.028	
"	Dr					.648			.648	
"	Ha			.714	.702				.708	
"	Mi (a)			1.065	.994				1.005	
"	Mi (b)						.720		.720	
"	Mi (c)						.690		.690	
"	Mi (d)			.729		.812		.883	.808	
"	Sm							.703	.703	
"	Sp			.975					.975	
"	We						.703		.703	
"	X						1.440		1.440	
Hemoglobin	Dr	1.00								1.000
"	Dr	1.35								1.350
"	Mo		.935							.935
"	Mo		.840							.840
"	Br		.792							.792
"	Br		.910							.910
"	Br		.885							.885
Averages		1.175	.872	.843	.895	.788	.888	.847	.854 ± .044*	.959 ± .070*

\* Standard error of the mean.

the occasional appearance of grease or of small amounts of adsorbed protein at the meniscus, indicate that the capillarity was not always the same at the different levels to which the meniscus was brought by the various temperature changes. The magnitude of the errors of capillarity, while not great in comparison with the total osmotic pressure, must be relatively large in proportion to the changes in pressure produced by changes in temperature. Consequently, one can draw conclusions from the data only on the basis of average values of the ratios, which may be expected to smooth out the unavoidable errors of the present method.

In spite of the errors, it would appear that temperature has a larger effect on the osmotic pressure of serum of some subjects than of others. It would appear, also, that the ratio is approximately the same over the various temperature ranges studied. The mean of the ratios for all determinations on serum is  $0.85 \pm .04$  (where  $\pm .04$  is the standard error of the mean). The mean for hemoglobin is  $0.96 \pm .07$ , the greater standard error being due, in part, to the relative paucity of data on hemoglobin as compared to

serum. The chances are about even that the true mean for serum may be as high as 0.89 and that the mean for hemoglobin may be as low as 0.89; consequently the difference between the observed means is not significant. The mean ratio for all the determinations, on serum and hemoglobin, is  $0.88 \pm .04$ . The standard deviation of the individual ratios about this mean is  $\pm 0.2$ .

Although the mean values of the ratios, as found, are not sufficiently accurate to justify a discussion of their significance in relation to the theory of solutions, they do indicate that, for practical purposes, no great error will be introduced by employing a ratio of 1.0 (as for the case of ideal solutions) in calculating the osmotic pressure of serum or hemoglobin at different temperatures. This assumption allows the use of the simple formula,  $\pi_2 = \pi_1 T_2/T_1$ , in which  $\pi$  is the osmotic pressure, and  $T$  is the absolute temperature. Employing this formula it is found, for example, that a correction of 6 per cent of the value of the osmotic pressure at 20° C. should be added to give the approximate pressure at body temperature.

References in the literature to previous studies

on the relation of temperature to the osmotic pressure of protein solutions are few, and the data have not been satisfactory. Martin (9), without publishing data, claimed to have found that Gay-Lussac's law was obeyed exactly. Adair (10) employed the thermodynamic relations based on this law in calculating the pressure of hemoglobin at 0° C. from some of his data which had been obtained in experiments at a higher temperature. Krogh and Nakazawa (11) failed to demonstrate any effect of temperature on the osmotic pressure of serum. Verney (12) was able to conclude that, while temperature has some effect on the osmotic pressure of serum, it is much less than that called for by the theory of ideal solutions.

TABLE II

*Showing that, within the limits of error of the method, the same equilibrium pressure is obtained on the same sample of serum with membranes of widely different permeabilities. Osmotic pressure in mm. of water at 20° C.*

Subject	Specific permeability ranges of membranes			
	1 to 3 $\times 10^{-8}$	6 to 12 $\times 10^{-8}$	20 to 57 $\times 10^{-8}$	200 to 300 $\times 10^{-8}$
Go	mm. H <sub>2</sub> O	mm. H <sub>2</sub> O	mm. H <sub>2</sub> O	mm. H <sub>2</sub> O
Ha		448	432	431
Sp	352	450	442	leak
		348	349	
			349	
			351	
Mi	363	366	366	
			364	
			360	
We (a)	385		412	
			400*	
			397	
			399	
			382	
			388	
We (b)	390	393	398	
			401	
			397	
			395	

\* Ultrafiltrate as outer solution.

The data shown in Table II provide direct evidence of the complete impermeability of the membranes to all fractions of the serum proteins. Within the limits of error of the method, the same pressure is observed when membranes are used

which are either much more (within limits) or much less permeable than the usual grade. It is probable that the membranes used in the standard osmotic pressure methods are much less permeable than the tightest membranes of our series (which did not usually require more than 8 to 12 hours for the attainment of equilibrium). Although some colloids may require tighter membranes, the specific permeability (3) range of  $20$  to  $40 \times 10^{-8}$  would appear to be ideal for blood serum, hemoglobin, and in all probability for many other types of protein solutions. Equilibrium, with such membranes, may be attained within 3 to 12 hours, and the average time required to carry out the whole determination is approximately 8 hours.

The possibility that some property of the membrane, other than its permeability, may affect the value of the equilibrium pressure has already been ruled out, in our opinion, by the work of Turner (13), who obtained the same values for the osmotic pressure of serum with membranes composed of a variety of materials.

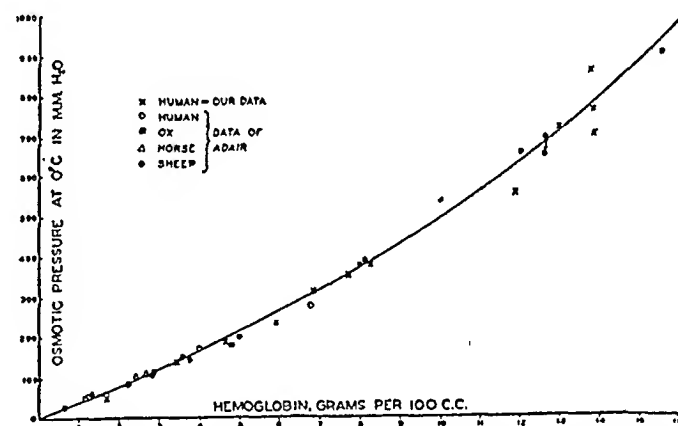


FIG. 1. Pressure-concentration graph of hemoglobin-salt solutions. Comparison of data obtained by the rapid method with values published by Adair. Curve, plotted from the formula,  $\pi = 37\text{Hb}/1 - 0.0254\text{Hb}$ , where  $\pi$  is the pressure in mm. of water at 0° C. and Hb is the concentration of hemoglobin in grams per 100 cc. of hemoglobin-Ringer solution of pH 8.0.

Figure 1 shows the relation of the osmotic pressure to the concentration of hemoglobin-Ringer solutions (prepared from the blood of four human subjects). It is to be noted that our values correspond very well with those obtained by Adair in his studies on solutions of hemoglobin, from various species. Adair (14) found that, in the presence of salts at a total concentration above 0.01 M., the osmotic pressure of hemoglobin is

independent of the pH, except at high concentrations of hemoglobin (above 6 to 10 per cent). Since we have employed the same pH which Adair used in most of his determinations (pH 8.00) and have made up our solutions with salts whose total concentration is considerably above 0.01 M. there is no reason to believe that the correspondence of our values with those of Adair can be attributable to chance. The pressure-concentration relations of hemoglobin solutions, as found by Adair, have previously been confirmed by others (2, 15).

It is probable that the degree of scattering of the points corresponding to the higher concentrations of hemoglobin is due in part to the relatively great effect of slight changes in salt concentration and of pH at concentrations of hemoglobin above 6 to 10 per cent (Adair (14)), and in part to the relative inaccuracy of the refractometric determination of the higher concentrations. In any case the data support the contention that the membranes used in our method are strictly semipermeable with respect to hemoglobin, and therefore, presumably, to serum proteins also.

The curve, drawn through the points of Figure 1 is plotted from the formula,  $\pi = 37\text{Hb}/1 - 0.0254\text{Hb}$ , which was obtained by Adair (14) from his data on the hemoglobin of sheep. The equation corresponds to the form  $\pi(v-b) = k$ , in which  $v = 100/\text{Hb}$ ,  $b = 2.54$  and  $k = 3700$ . The pressure is expressed in mm. of water and the concentration, Hb, in grams per 100 cc. Adair finds that this equation expresses the relation of concentration to pressure (at 0° C.) up to 20 per cent hemoglobin. Values of the osmotic pressure calculated by this formula may conveniently be employed for a comparison with observed values in tests which may be carried out in any laboratory to check the accuracy of an osmotic pressure method. Such tests may be carried out at any convenient temperature above 0° C., for the corresponding pressure values may then be corrected to 0° C. by the application of the equation,  $\pi_2 = \pi_1 T_2/T_1$ , which, as our studies have demonstrated, is applicable to hemoglobin solutions.

#### SUMMARY

Critical tests applied to the procedures in use in this laboratory for the determination of the

osmotic pressure of proteins in biological fluids indicate that the method gives reliable results:

1. Serum is shown to remain osmotically stable to variations in temperature from 10° C. to 37° C. for a sufficient time to allow the determination to be carried out. Variations in temperature, within this range, produce changes in the osmotic pressure which are reversible and which are of the magnitude to be expected from the theory of ideal solutions.

2. The membranes employed are completely impermeable to the serum proteins, for their permeability may be either decreased or increased, over a wide range of specific permeability values, without affecting the value of the observed osmotic pressure.

3. The osmotic pressure of hemoglobin solutions, as determined by the rapid method, are identical, within the limits of error, with values established by others, who have used the "slow" methods hitherto considered essential for accurate studies. The practicability of the use of hemoglobin solutions, for the standardization of osmotic pressure methods in all laboratories is emphasized.

4. The rapid method, standardized as indicated, appears to be sufficiently reliable to warrant its use in many types of investigations of the osmotic properties of proteins.

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# THE EXCHANGE OF LIPIDS IN THE UMBILICAL CIRCULATION AT BIRTH

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Pregnancy in women has been shown by Boyd (1) to be accompanied by a lipemia involving certain definite changes in the lipid concentration of the red blood cells and blood plasma. Correlating this with earlier work the conclusion was reached that the increase in the concentration of blood lipids commences about midway in the nine months of gestation. Fat metabolism in the fetus also becomes active about the mid-point of pregnancy as indicated by increasing amounts of lipids being laid down in the growing offspring (2). The time relationship of these two processes suggests that they may be related.

To prove or disprove this possible relation was the purpose of the present investigation. As a working hypothesis it was suggested that as the lipid concentration in the maternal blood stream increases, the placenta passes more of these substances on to umbilical blood from whence they are absorbed in increasing amounts by the fetus. This theory requires proof (a) that lipids are absorbed from umbilical blood by the fetus, and (b) that lipids are added to umbilical blood by the placenta. The placenta of the rabbit and rat has been shown in recent years to be "permeable" to lipids by Bickenbach and Rupp (14), Sinclair (15) and Chaikoff and Robinson (16). By analogy the human placenta has been assumed to be "permeable" also. Direct proof of the "permeability" of the human placenta to lipids is lacking and it should be recalled that the structure of the human placenta differs from that of the rat and the rabbit. In fact Slemons and Stander (3) and many others as reviewed by Needham (2), Mayer (4), Schlossmann (5), etc., believe that the human placenta does not permit the passage of fats to the fetal circulation because they found that the lipid concentration of blood on the maternal side of the placenta was always higher than

on the fetal side. We have repeated this experiment and obtained similar results but we are inclined to agree with Needham (2) that it does not prove the human placenta to be "impermeable" to lipids. Hence we devised experiments of a different nature which could be applied with safety both to mother and child.

It was decided that the first requisite was to determine whether or not the fetus was capable of absorbing lipids from umbilical blood. Obviously if lipids were not taken up by the fetus as blood passed through it, then there was no need for the placenta to pass them on from the maternal blood stream. On the other hand if lipids were absorbed by the fetus from umbilical blood, then presumably the placenta must replenish the lost supply and such would be circumstantial evidence in favor of the "permeability" of the human placenta to lipids. When the term permeability is applied to lipids we do not mean simple diffusion as in the case of electrolytes. How the colloidal and insoluble lipid molecules pass through animal membranes is not understood but possibly it is through the intermediate formation of the semi-water soluble phospholipids.

## PART A. FAT ABSORPTION BY THE HUMAN FETUS AT BIRTH

The procedure developed consisted in obtaining samples of blood from the umbilical vein and an umbilical artery immediately after parturition and comparing the lipid composition of each. The umbilical vein (Figure 1) carries blood from the placenta to the fetus while the umbilical arteries return blood from the fetus to the placenta after it has passed through the embryo. By comparing the lipid content of blood entering the fetus by the umbilical vein with the lipid content of blood leaving the fetus by the umbilical arteries, it may be found what lipids, if any, are added to or removed from the blood in its passage through the fetus.

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This method was used as early as 1884 by Cohnstein and Zuntz (6) to investigate the physiology of the fetus. More recently it has been employed by Arstamianz (7), Blair-Bell et al. (8) and Naeslund (9). As far as the authors have been able to ascertain, no previous reports have appeared on the lipid composition of blood in both vessels. In 1913 Grigaut (10) reported in the course of a thesis that in three cases he found 11 to 12 per cent more cholesterol in the vein than in the artery and this was confirmed in 1926 by Arstamianz (7). Of the other lipids, ester cholesterol, free cholesterol, phospholipids, neutral fats, etc., apparently nothing has been reported.

All samples of blood were obtained from patients in the delivery rooms of the Strong Memorial Hospital. No attempt was made to select cases in regard to age, nationality, weight or parity; each patient was a normal, healthy woman at full term pregnancy and in labor. Since most obstetrical patients enter the maternity hospital in active labor, it was not possible to regulate the previous habitual diet of the patients nor to control the length of time between delivery and when the last meal was taken. Labor lasts from 15 to 24 hours and during this time most patients consume very little food so that the results may be considered approximately fasting values. To assure no harm to the health of the new born child, blood was not taken from the fetal end of the cord (umbilical artery) when the delivered infant

that all the new born infants were large and well nourished.

With the patient in the expulsive stage of labor, one of us "scrubbed in" on the delivery and was handed the child by the accoucher immediately after it had been removed from the uterus. The umbilical cord was clamped and cut immediately after delivery instead of waiting for the cord to stop pulsating in the orthodox manner. Cessation of pulsation in the umbilical cord signified the contraction of the umbilical arteries and under such circumstances it was found difficult to obtain a sample of blood from these vessels. For the same reason the new born child was quickly transferred to a heated crib and the cord stump (cut about 6 to 8 inches from the child) wrapped in warm towels.

The clamp holding the cord stump was then released and the cut end of the cord searched for an umbilical artery which was readily recognized by the pulsating spurts of blood emitted from it. The remaining cross section of the cord was clamped, excluding the artery from which blood was allowed to pump into a flask containing a small amount of half saturated sodium citrate solution. Ten to 20 cc. of blood were so obtained in about 5 minutes. A sample of venous blood was then taken from the maternal end of the cord with the placenta still attached in utero. The latter sample was considered to represent the venous blood entering the fetus. The contraction of the uterus was assumed to have little effect upon the lipid content of venous blood, an assumption which was substantiated in part by the finding of similar results in a case of caesarean section in which the uterus was not contracted. It was later found that the lipid concentration of venous blood slowly increases after the cord is clamped so that this sample was obtained as quickly as possible after delivery. The samples of whole blood from the artery and vein were then extracted and analysed for their lipid constituents using the Bloor oxidative micro-procedures as modified by Boyd (11).

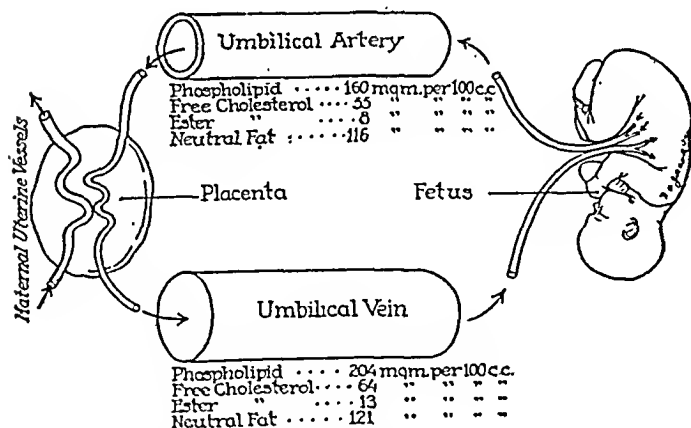


FIG. 1. DIAGRAM REPRESENTING THE EXCHANGE OF LIPIDS BETWEEN THE HUMAN FETUS AND PLACENTA AT THE TIME OF PARTURITION.

weighed under 3,000 grams and most of the infants were large, weighing between 3,500 and 4,500 grams. It is important to remember for the interpretation of the data to be presented below

## RESULTS

On each sample of blood the ten lipid values given by Boyd (11) were determined but for the sake of brevity and clarity only the essential values will be reported in detail. In Tables I to IV

are listed the individual values respectively for phospholipid, free cholesterol, ester cholesterol and neutral fat. The remaining values (total lipid, total fatty acids, etc.) may be calculated from those given in the manner described by Boyd (11).

Fetal blood is known to contain three groups of lipids, namely phospholipids, neutral or glycerol fats and cholesterol and its esters. Phospholipids may be regarded as esters of fatty acids in combination with glycerol, a nitrogenous base or bases and phosphoric acid. The latter radicles impart to these substances the property of being partially soluble in water in which they form colloidal solutions. They also have a smaller molecular weight than most other lipids. For these reasons and from various experiments on fat absorption they are usually regarded as functioning in the transport of fatty acids.

TABLE I

*Comparison of the phospholipid content of whole blood from the umbilical vein and the umbilical artery at birth*

Unit number	Umbilical vein (blood going to fetus)	Umbilical artery (blood coming from fetus)	Phospholipid absorbed by fetus
	<i>mgm. per 100 cc.</i>	<i>mgm. per 100 cc.</i>	<i>mgm. per 100 cc.</i>
16033	300	196	104
81576	250	223	27
75842	223	170	53
80482	219	183	36
70067	218	196	22
46488	213	83	130
56521	197	142	55
59820-A	192	83	109
59820-B	190	162	28
82597	185	163	22
53777	182	153	29
61916	180	170	10
80368	175	173	2
60631	168	140	28
85939	162	161	1

The *phospholipid* (Table I) content of venous blood going to the fetus was found to range from 162 to 300 mgm. per cent with a mean value of 204 mgm. per cent. Blood leaving the fetus by the umbilical artery contained a lower range of values, 83 to 223 mgm. per cent and an average of 160 mgm. per cent; in each case the value in arterial blood was lower than in venous blood. If these values may be taken to represent the actual concentrations in blood entering and leaving the fetus then it may be calculated that the

fetus absorbed from 1 to 130 mgm. of phospholipids from every 100 cc. of blood. The mean amount of phospholipids absorbed would be 44 mgm. per 100 cc. or 22 per cent of the phospholipids in blood sent to the fetus by the placenta. Considered in this light the results indicate that the fetus absorbs large amounts of phospholipids from umbilical blood.

TABLE II

*Comparison of the free cholesterol content of whole blood from the umbilical artery and umbilical vein at birth*

Unit number	Umbilical vein (blood going to fetus)	Umbilical artery (blood coming from fetus)	Free cholesterol absorbed by fetus	Free cholesterol given up by fetus
	<i>mgm. per 100 cc.</i>	<i>mgm. per 100 cc.</i>	<i>mgm. per 100 cc.</i>	<i>mgm. per 100 cc.</i>
61916	88	68	20	0
75842	74	67	7	0
16033	72	71	1	0
81576	70	65	5	0
70067	69	68	1	0
46488	64	39	25	0
85939	63	53	10	0
59820-A	62	39	23	0
53777	60	75	0	15
59820-B	59	51	8	0
80482	58	55	3	0
56521	56	37	19	0
80368	56	58	0	2
82597	55	37	18	0
60631	51	46	5	0

*Free cholesterol*, Table II, exhibited changes similar to phospholipids. In all except two cases blood from the vein contained more free cholesterol than blood from the artery. In the former vessel the range was 51 to 88 mgm. per cent while in the latter it was 37 to 75 mgm. per cent giving an average of 64 mgm. per cent for the vein and 55 for the artery. Thus the fetus may be considered to have absorbed from 0 to 25 mgm. of free cholesterol per 100 cc. of blood, a mean absorption of 9 mgm. or 14 per cent of the free cholesterol going to the fetus from the placenta. It may thus be concluded that the fetus also absorbs free cholesterol though in appreciably smaller amounts than phospholipids. Cholesterol has the property of combining with fatty acids to produce cholesterol esters, substances which Bloor believes may act as transporters of fatty acids. That part of the total cholesterol which is united with fatty acids is designated ester cholesterol.



TABLE III

*Comparison of the ester cholesterol content of whole blood from the umbilical vein and the umbilical artery at birth*

Unit number	Umbilical vein (blood going to fetus)	Umbilical artery (blood coming from fetus)	Ester cholesterol absorbed by fetus	Ester cholesterol given up by fetus
	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.
56521	49	16	33	0
80368	34	13	21	0
70067	28	7	21	0
46488	26	6	20	0
82597	19	5	14	0
53777	15	5	10	0
59820-A	10	2	8	0
75842	6	6	0	0
16033	4	0	4	0
80482	4	13	0	9
81576	1	14	0	13
61916	0	21	0	21
85930	0	15	0	15
60631	0	1	0	1
59820-B	0	3	0	3

*Ester cholesterol*, Table III, was found lower in the artery in 8 cases, lower in the vein in 6 cases and in 1 case was the same in both vessels. There appeared to be no relation between these changes until they were tabulated as in Table III according to the height of ester cholesterol in venous blood. It then became obvious that the more ester cholesterol in venous blood going to the fetus the more absorbed by the fetus. When the concentration of ester cholesterol in the umbilical vein fell below about 10 mgm. per cent then the fetus not only ceased to absorb this lipid but actually added some to the blood. This may conceivably represent an attempt on the part of the fetus to maintain a certain level of ester cholesterol in the blood. The results indicate that the fetus absorbs ester cholesterol providing there is more than a certain minimum concentration (10 mgm. per cent) in the blood.

*Neutral fat*, Table IV, behaved in a distinctly different manner from the other three lipids. In 9 cases there was more neutral fat in the venous blood and in 6 cases more in the arterial blood. No reason for the variation could be found such as differences in the concentration in venous blood, weight of fetus, etc. Very little is known about the function of neutral fat in blood: Boyd has shown that its concentration in blood is subject to marked variation under normal conditions

(11) and that it is more than doubled in value in plasma at the end of pregnancy (1). From the present results neutral fat may apparently be either absorbed or given up by the fetus. Its mean

TABLE IV

*Comparison of the neutral fat content of whole blood from the umbilical vein and umbilical artery at birth*

Unit number	Umbilical vein (blood going to fetus)	Umbilical artery (blood coming from fetus)	Neutral fat absorbed by fetus	Neutral fat given up by fetus
	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.
81576	195	263	0	68
16033	193	206	0	13
82597	186	196	0	10
85930	179	136	43	0
61916	172	64	108	0
80482	163	96	67	0
59820-B	122	104	18	0
60631	94	82	12	0
53777	94	138	0	44
80368	92	150	0	58
75842	88	83	5	0
59820-B	76	50	26	0
70067	66	58	8	0
46488	61	58	3	0
56521	31	57	0	26

values together with corresponding values for the other lipids have been given in Figure 1; the data given in this figure may be considered as a short summary of the results above.

Of particular interest among the cases studied were those of Unit Number 59820-A and -B. These were double ovum twins of the same sex and weight. One might expect to find the same amount of lipids going to and coming from each. Yet this was not the case. Twin A absorbed more phospholipids, more free cholesterol, more ester cholesterol and more neutral fat than Twin B. These findings in conjunction with the wide range of values for each lipid as given in Tables I to IV indicate that there is considerable variation from one fetus to another in respect to the absorption of lipids from umbilical blood.

#### *The composition of fatty acids in fetal blood*

In six cases iodine numbers were determined on the fatty acids of umbilical vein plasma. The values obtained for the iodine number of total fatty acids were 60, 66, 72, 76, 88 and 119 or a mean of 80. The average iodine number of total

fatty acids in the plasma of adult normal and pregnant women is about the same, namely 86 (1). This indicates that the fatty acids of blood plasma are probably similar in composition in the fetus as in the adult. The iodine numbers of phospholipid fatty acids from the same plasma of fetal venous blood were found to be 53, 58, 96, 94, 126 and 149 or an average of 96, somewhat lower than the corresponding value for adults, 120 (1). The phospholipids of fetal blood therefore probably contain more saturated fatty acids than phospholipids of adult plasma.

In two cases a sufficient amount of arterial blood was obtained to enable a comparison of iodine numbers in venous and arterial blood. In both cases the iodine number of phospholipid fatty acids was higher in arterial than venous blood, the values being respectively 176 to 149 and 142 to 58. The results suggest that the fetus has a distinct preference for saturated fatty acids. Furthermore, the phospholipid fraction of arterial plasma gave no precipitation from ethereal solution with alcohol whereas a marked cloudiness developed in the solution of venous phospholipids. Since alcohol precipitates cephalin from ethereal solution and does not affect lecithin these preliminary tests suggest that the fetus has an avidity for cephalin in particular. Cephalin is found in appreciable amounts in brain and nerve tissue.

The results presented in Part A indicate clearly that whole blood collected from the umbilical vein immediately after delivery contains a greater concentration of most lipids than blood collected from the umbilical artery of the infant also collected immediately after delivery. Phospholipids are markedly lower in value in arterial blood, free cholesterol somewhat less so, ester cholesterol usually lower but depending on the concentration in the vein and neutral fat varying one way or the other. When the pathway of the fetal circulation, as depicted in Figure 1, is taken into consideration it appears reasonable to interpret these results as signifying an absorption of the first three of these lipids by the fetus from umbilical blood. The fact that all lipids were not reduced to the same relative extent in arterial blood indicates that the results are not due to a simple dilution of venous blood in its passage through the fetus. Since the samples of blood were collected

immediately after the cord had been cut it is probable that fat was absorbed by the fetus at the same rate as when the umbilical circulation was intact. We may therefore conclude that the human fetus at birth absorbs from umbilical blood phospholipids, free cholesterol and cholesterol esters and occasionally neutral fat also.

By analysing the results more closely, it is possible to arrive at an approximate idea of the *amount* of lipids being absorbed by the fetus per day. It will be recalled that blood was taken from the umbilical artery within 5 minutes of the time that the cord was clamped and cut. The fetus thus had 5 minutes in which to absorb lipids from its blood stream before all of the arterial sample of blood was secured. If we assume that no lipids were added to the blood by the fetus in this interval then we may calculate the total amount of lipids absorbed in 5 minutes, in an hour or in a day. The concentration of lipids in arterial blood subtracted from the concentration in the vein will thus roughly indicate the amount of lipids absorbed from 100 cc. of blood in five minutes. Multiplying this value by  $2\frac{1}{2}$  will give the total amount of lipid absorbed from the entire fetal blood stream which is usually reckoned as 250 cc. in the average infant.

Applying these calculations to phospholipid values it may be found that an average large well nourished human infant at delivery absorbs 44 mgm. of phospholipids per 100 cc. of blood per 5 minutes or approximately 30 grams per 250 cc. per 24 hours. Although this is only an approximation it is probably fairly correct: there are certain factors which would lower it and certain which would increase it. The important point is that it indicates that the human fetus at birth absorbs very large quantities of this lipid; 30 grams represents three times as much phospholipid as the maternal blood stream contains at any one time. In a similar manner it may be calculated that free cholesterol is absorbed at the average rate of 6 to 7 grams per day and cholesterol esters (ester cholesterol plus cholesterol ester fatty acids) at about the same rate. In all, 40 to 50 grams of these lipids are absorbed from umbilical blood per day by the human fetus. Part of this fat is no doubt used as fuel in metabolism and part stored in the fat depots.

As previously stated, it is probable that lipid

absorption by the fetus is similar just before the cord is cut to what it is just after. Before the cord is clamped and cut, blood travels in a closed circuit from the placenta to the fetus and back to the placenta. If the fetus removes lipids from this blood, then lipids must be added by the placenta to make up for the loss. The experimental data given may be taken to prove this fact. By referring to Figure 1 it may be seen that arterial blood may be regarded as blood entering the placenta and venous blood as blood leaving the placenta. Since venous blood has been shown to contain increased amounts of most lipids, it may be concluded that these substances are added to umbilical blood in its passage through the placenta. Additional evidence that the placenta actually does add these lipids to umbilical blood will now be offered.

#### PART B. THE ADDITION OF LIPIDS TO UMBILICAL BLOOD BY THE PLACENTA AT BIRTH

When the human fetus is delivered, the umbilical cord is clamped and cut. For a period of from 10 to 30 minutes, the placenta remains attached to the uterine wall before separating and being expelled by the recurrence of uterine contractions. During most of this interval the placental and maternal blood remain in the same relationship as in the latter part of pregnancy. Hence if lipids are added to the placental blood by the maternal blood stream, they will continue to do so until the placenta separates. The umbilical cord being clamped, circulation of blood through the placenta and fetus has ceased and any lipids which are added to umbilical blood will not be removed by the fetus. As a result these lipids will accumulate in placental blood and raise the concentration of lipids there. Thus, if a sample of umbilical blood is obtained immediately after the child is delivered when the cord is cut and a second sample obtained 10 to 30 minutes later when the placenta separates from the uterine wall, there should be a greater concentration of lipids in the second sample than in the first providing the premise is correct that the placenta adds lipids to umbilical blood.

Such a procedure was adopted. Twenty-five to 50 cc. of blood (Sample 1) were taken from the umbilical vein immediately after the cord had been cut. The uterus was then palpated through the

mother's abdomen until the placenta was noted to separate from the uterine wall. Then the uterus was compressed and as much blood as possible—usually 50 to 75 cc.—collected from the umbilical vein in another flask (Sample 2). Each sample of blood was separately extracted and analysed for its lipid content.

TABLE V

*Variation in the phospholipid content of whole blood from the umbilical vein between the time of delivery and 10 to 30 minutes later at the time the placenta separates from the uterus*

Case number	At delivery	At placental separation	Phospholipid added by placenta	Phospholipid removed by placenta
	<i>mgm. per 100 cc.</i>	<i>mgm. per 100 cc.</i>	<i>mgm. per 100 cc.</i>	<i>mgm. per 100 cc.</i>
1	162	192	30	0
2	165	184	19	0
3	167	197	30	0
4	168	219	41	0
5	175	204	29	0
6	182	236	52	0
7	185	201	16	0
8	190	163	0	27
9	192	253	61	0
10	197	212	15	0
11	213	226	13	0
12	218	222	4	0
13	219	225	6	0
14	223	210	0	13

*Phospholipid*, Table V, again exhibited the most consistent and extensive changes. In twelve out of fourteen cases, Sample 2 contained more phospholipids than Sample 1. At delivery the phospholipid concentration in whole blood from the umbilical vein varied between 162 and 223 mgm. per cent with a mean of 190 mgm. per cent. At the time of placental separation half an hour later, the range was 163 to 236 mgm. per cent with a mean of 210 mgm. per cent and an average addition of 20 mgm. of phospholipid per 100 cc. of blood. Since, as will be shown, the other lipids were not increased at the same rate, the increase in phospholipid cannot have been due simply to the placenta removing water from the umbilical blood. It appears logical to conclude that these results indicate phospholipid is actually added to umbilical blood between the time of delivery and the time of placental separation, and it is probable that such an addition also occurs before delivery. That phospholipid is added to umbilical blood by the placenta explains how phospholipid is restored to umbilical blood when removed by the fetus.

TABLE VI

*Variation in the free cholesterol content of whole blood from the umbilical vein between the time of delivery and 10 to 30 minutes later at the time the placenta separates from the uterus*

Case number	At delivery	At placental separation	Free cholesterol added by placenta	Free cholesterol removed by placenta
	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.
1	49	57	8	0
2	50	66	16	0
3	51	51	0	0
4	55	67	12	0
5	56	61	15	0
6	58	72	14	0
7	59	53	0	6
8	60	69	9	0
9	62	78	16	0
10	63	67	4	0
11	64	65	1	0
12	66	72	6	0
13	69	66	0	3
14	74	73	0	1

*Free cholesterol*, Table VI, was also more concentrated in Sample 2 in the majority of cases. The average value for free cholesterol at delivery was 60 mgm. per cent and at placental separation 65 mgm. per cent. In 72 per cent of cases Sample 2 contained more free cholesterol than Sample 1, in 7 per cent there was no change and in 21 per cent free cholesterol was lower in the second sample of blood. Individual differences between the two samples were small as would be expected. The results may be interpreted as signifying that the human placenta adds free cholesterol to umbilical blood.

*Ester cholesterol*, Table VII, behaved in a manner similar to free cholesterol. The mean value for ester cholesterol at delivery was 16 mgm. per cent and at placental separation 19 mgm. per cent. In 64 per cent of cases ester cholesterol was increased in Sample 2, in 7 per cent unchanged and in 29 per cent decreased. It may therefore be concluded that cholesterol esters are often added to umbilical blood by the placenta.

*Neutral fat*, Table VIII, again exhibited marked variation. In 50 per cent of cases Sample 2 contained more neutral fat than Sample 1, in 43 per cent there was less and in 7 per cent both samples contained the same amount. The average value at birth was 108 mgm. per cent and at placental separation 88 mgm. per cent. The

results indicate that neutral fat may be either added to or removed from umbilical blood by the placenta. The factor influencing the direction of the exchange was not found.

TABLE VII

*Variation in the ester cholesterol content of whole blood from the umbilical vein between the time of delivery and 10 to 30 minutes later at the time the placenta separates from the uterus*

Case number	At delivery	At placental separation	Ester cholesterol added by placenta	Ester cholesterol removed by placenta
	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.
1	0	0	0	0
2	0	19	19	0
3	0	21	21	0
4	4	15	11	0
5	6	17	11	0
6	10	1	0	9
7	12	15	3	0
8	15	13	0	2
9	18	21	3	0
10	20	27	7	0
11	26	30	4	0
12	28	30	2	0
13	34	32	0	2
14	49	27	0	22

TABLE VIII

*Variation in the neutral fat content of whole blood from the umbilical vein between the time of delivery and 10 to 30 minutes later at the time the placenta separates from the uterus*

Case number	At delivery	At placental separation	Neutral fat added by placenta	Neutral fat removed by placenta
	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.
1	31	31	0	0
2	47	4	0	43
3	61	36	0	25
4	66	74	8	0
5	76	0	0	76
6	88	92	4	0
7	92	102	10	0
8	94	23	0	71
9	94	157	63	0
10	108	100	0	8
11	122	36	0	86
12	163	181	18	0
13	179	195	16	0
14	186	196	10	0

The results of Part B demonstrate that blood from the umbilical vein at placental separation contains more phospholipids in practically 100 per cent of cases, more free cholesterol in about 75 per cent of cases, more cholesterol esters in about

absorption by the fetus is similar just before the cord is cut to what it is just after. Before the cord is clamped and cut, blood travels in a closed circuit from the placenta to the fetus and back to the placenta. If the fetus removes lipids from this blood, then lipids must be added by the placenta to make up for the loss. The experimental data given may be taken to prove this fact. By referring to Figure 1 it may be seen that arterial blood may be regarded as blood entering the placenta and venous blood as blood leaving the placenta. Since venous blood has been shown to contain increased amounts of most lipids, it may be concluded that these substances are added to umbilical blood in its passage through the placenta. Additional evidence that the placenta actually does add these lipids to umbilical blood will now be offered.

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When the human fetus is delivered, the umbilical cord is clamped and cut. For a period of from 10 to 30 minutes, the placenta remains attached to the uterine wall before separating and being expelled by the recurrence of uterine contractions. During most of this interval the placental and maternal blood remain in the same relationship as in the latter part of pregnancy. Hence if lipids are added to the placental blood by the maternal blood stream, they will continue to do so until the placenta separates. The umbilical cord being clamped, circulation of blood through the placenta and fetus has ceased and any lipids which are added to umbilical blood will not be removed by the fetus. As a result these lipids will accumulate in placental blood and raise the concentration of lipids there. Thus, if a sample of umbilical blood is obtained immediately after the child is delivered when the cord is cut and a second sample obtained 10 to 30 minutes later when the placenta separates from the uterine wall, there should be a greater concentration of lipids in the second sample than in the first providing the premise is correct that the placenta adds lipids to umbilical blood.

Such a procedure was adopted. Twenty-five to 50 cc. of blood (Sample 1) were taken from the umbilical vein immediately after the cord had been cut. The uterus was then palpated through the

mother's abdomen until the placenta was noted to separate from the uterine wall. Then the uterus was compressed and as much blood as possible—usually 50 to 75 cc.—collected from the umbilical vein in another flask (Sample 2). Each sample of blood was separately extracted and analysed for its lipid content.

TABLE V

*Variation in the phospholipid content of whole blood from the umbilical vein between the time of delivery and 10 to 30 minutes later at the time the placenta separates from the uterus*

Case number	At delivery	At placental separation	Phospholipid added by placenta	Phospholipid removed by placenta
	<i>mgm. per 100 cc.</i>	<i>mgm. per 100 cc.</i>	<i>mgm. per 100 cc.</i>	<i>mgm. per 100 cc.</i>
1	162	192	30	0
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11	213	226	13	0
12	218	222	4	0
13	219	225	6	0
14	223	210	0	13

*Phospholipid*, Table V, again exhibited the most consistent and extensive changes. In twelve out of fourteen cases, Sample 2 contained more phospholipids than Sample 1. At delivery the phospholipid concentration in whole blood from the umbilical vein varied between 162 and 223 mgm. per cent with a mean of 190 mgm. per cent. At the time of placental separation half an hour later, the range was 163 to 236 mgm. per cent with a mean of 210 mgm. per cent and an average addition of 20 mgm. of phospholipid per 100 cc. of blood. Since, as will be shown, the other lipids were not increased at the same rate, the increase in phospholipid cannot have been due simply to the placenta removing water from the umbilical blood. It appears logical to conclude that these results indicate phospholipid is actually added to umbilical blood between the time of delivery and the time of placental separation, and it is probable that such an addition also occurs before delivery. That phospholipid is added to umbilical blood by the placenta explains how phospholipid is restored to umbilical blood when removed by the fetus.

TABLE VI

*Variation in the free cholesterol content of whole blood from the umbilical vein between the time of delivery and 10 to 30 minutes later at the time the placenta separates from the uterus*

Case number	At delivery	At placental separation	Free cholesterol added by placenta	Free cholesterol removed by placenta
	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.
1	49	57	8	0
2	50	66	16	0
3	51	51	0	0
4	55	67	12	0
5	56	61	15	0
6	58	72	14	0
7	59	53	0	6
8	60	69	9	0
9	62	78	16	0
10	63	67	4	0
11	64	65	1	0
12	66	72	6	0
13	69	66	0	3
14	74	73	0	1

*Free cholesterol*, Table VI, was also more concentrated in Sample 2 in the majority of cases. The average value for free cholesterol at delivery was 60 mgm. per cent and at placental separation 65 mgm. per cent. In 72 per cent of cases Sample 2 contained more free cholesterol than Sample 1, in 7 per cent there was no change and in 21 per cent free cholesterol was lower in the second sample of blood. Individual differences between the two samples were small as would be expected. The results may be interpreted as signifying that the human placenta adds free cholesterol to umbilical blood.

*Ester cholesterol*, Table VII, behaved in a manner similar to free cholesterol. The mean value for ester cholesterol at delivery was 16 mgm. per cent and at placental separation 19 mgm. per cent. In 64 per cent of cases ester cholesterol was increased in Sample 2, in 7 per cent unchanged and in 29 per cent decreased. It may therefore be concluded that cholesterol esters are often added to umbilical blood by the placenta.

*Neutral fat*, Table VIII, again exhibited marked variation. In 50 per cent of cases Sample 2 contained more neutral fat than Sample 1, in 43 per cent there was less and in 7 per cent both samples contained the same amount. The average value at birth was 108 mgm. per cent and at placental separation 88 mgm. per cent. The

results indicate that neutral fat may be either added to or removed from umbilical blood by the placenta. The factor influencing the direction of the exchange was not found.

TABLE VII

*Variation in the ester cholesterol content of whole blood from the umbilical vein between the time of delivery and 10 to 30 minutes later at the time the placenta separates from the uterus*

Case number	At delivery	At placental separation	Ester cholesterol added by placenta	Ester cholesterol removed by placenta
	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.
1	0	0	0	0
2	0	19	19	0
3	0	21	21	0
4	4	15	11	0
5	6	17	11	0
6	10	1	0	9
7	12	15	3	0
8	15	13	0	2
9	18	21	3	0
10	20	27	7	0
11	26	30	4	0
12	28	30	2	0
13	34	32	0	2
14	49	27	0	22

TABLE VIII

*Variation in the neutral fat content of whole blood from the umbilical vein between the time of delivery and 10 to 30 minutes later at the time the placenta separates from the uterus*

Case number	At delivery	At placental separation	Neutral fat added by placenta	Neutral fat removed by placenta
	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.
1	31	31	0	0
2	47	4	0	43
3	61	36	0	25
4	66	74	8	0
5	76	0	0	76
6	88	92	4	0
7	92	102	10	0
8	94	23	0	71
9	94	157	63	0
10	108	100	0	8
11	122	36	0	86
12	163	181	18	0
13	179	195	16	0
14	186	196	10	0

The results of Part B demonstrate that blood from the umbilical vein at placental separation contains more phospholipids in practically 100 per cent of cases, more free cholesterol in about 75 per cent of cases, more cholesterol esters in about

two thirds of the cases and more neutral fat in only one half the cases than at the time of delivery. These results consistently agree with the findings in Part A. Phospholipids are of paramount importance in the exchange of lipids between the placenta and the fetus: they are added to umbilical blood by the placenta and absorbed by the fetus. Free cholesterol is also absorbed by the fetus but in smaller amounts; and in only three quarters of the cases studied could the placenta be shown to add free cholesterol to umbilical blood. Ester cholesterol is absorbed by the fetus providing there is more than about 10 mgm. per cent in its blood; when the concentration falls below 10 mgm. per cent the placenta adds large amounts but if the concentration is above 10 mgm. per cent the placenta may either add or subtract a few mgm. Apparently both the placenta and the fetus take part in keeping the value of ester cholesterol in umbilical blood at about 10 mgm. per cent. Neutral fat may be either absorbed or given up both by the placenta and the fetus; what factors govern the direction of the exchange are not apparent from the results to date.

#### *The rôle of the white blood cells*

In view of some of the older theories which ascribed to blood leukocytes an important function in fat absorption from the intestine, it was decided to investigate the lipid content of these cells in the fetal circulation. The methods of separation and analysis used were those of Boyd (12).

In 11 cases sufficient blood was obtained from the umbilical vein to perform a complete analysis on the leukocytes and the results are given in Table IX. The outstanding difference between the lipid content of leukocytes in the fetal blood and those of adult blood as previously reported (12) is that the fetal white cells contain smaller amounts of all the various lipid constituents, approximately one half the values in adults. The difference was less marked though still distinctly lower than the concentration of lipids in the leukocytes of pregnant women in whom the values are somewhat lower than in non-pregnant women (13).

Differential leukocyte counts in fetal and infant blood have shown that there is a greater percentage of lymphocytes in the young, as much as 50

per cent of the total leukocytes. The low lipid content is possibly related to this greater proportion of lymphocytes. The blood leukocytes in a case of chronic lymphatic leukemia studied by one of us (E. M. B.) gave evidence of low lipid values and the leukocytes were composed chiefly of lymphocytes.

TABLE IX

*The lipid composition of the white blood cells in umbilical vein blood at birth*

Case number	Total lipid	Composition of total lipid				
		Neutral fat	Cholesterol			Phospholipid
			Total	Ester	Free	
	mgm. per 100 grams	mgm. per 100 grams	mgm. per 100 grams	mgm. per 100 grams	mgm. per 100 grams	mgm. per 100 grams
1	1540	660	170	0	179	710
2	1105	394	239	0	243	472
3	932	0	431	280	151	313
4	845	158	185	63	122	460
5	793	112	156	74	82	475
6	759	0	201	9	192	552
7	719	82	170	0	172	467
8	691	0	163	48	115	496
9	691	0	196	27	169	477
10	686	0	174	0	174	512
11	566	41	174	58	116	342
Mean	848	132	205	51	156	480

In only 4 cases was sufficient blood obtained from the umbilical artery to permit a lipid analysis of the white blood cells. All the values found were within the same range as the venous leukocytes. Comparing the lipid composition of the venous leukocytes to that of the arterial in the same infant, each lipid was found changed after passing through the fetus but in the 4 cases studied no consistent variation for any lipid could be established. Nor was there any consistent difference in the lipid values of the venous leukocytes between the time of delivery and the time of placental separation. In fact neither by comparing individual differences nor general trends and means could any evidence be obtained that the blood leukocytes aided the transfer of lipids between the placenta and the fetus.

#### SUMMARY

Evidence has been presented that certain lipids are added to umbilical blood by the placenta and removed or absorbed by the fetus.

(1) Whole blood from the umbilical artery at



birth contained 22 per cent less phospholipids and 14 per cent less free cholesterol than whole blood from the umbilical vein. Ester cholesterol was also lower in arterial blood but only when venous blood contained over 10 mgm. per cent of this lipid. Neutral fat was lower in about half the cases and higher in the other half. The results were considered to signify that phospholipid and free cholesterol are regularly absorbed by the human fetus from umbilical blood at birth, ester cholesterol providing that there is sufficient (over 10 mgm. per cent) to be absorbed while neutral fat may be either absorbed or given up. It was estimated that over 40 grams, 75 per cent of which is phospholipids, of these substances are absorbed in 24 hours by an average large, well nourished human fetus at birth.

(2) Whole blood which lies in the placenta between the time that the cord is clamped and the time that the placenta separates from the uterine wall was found to acquire additional amounts of phospholipids in 12 out of 14 cases, free cholesterol in three quarters of the cases, ester cholesterol in two thirds of the cases and neutral fat in half the cases. It was concluded that the placenta adds all of these substances to umbilical blood and may remove some of them, especially neutral fat. Phospholipids, free cholesterol and ester cholesterol therefore pass in general in one direction, namely from the placenta to the fetus, while neutral fat may pass in either direction.

(3) The composition of fatty acids in plasma from the umbilical vein were found similar, in respect to their iodine number, to those of adult plasma except phospholipid fatty acids which are apparently more saturated in the fetal circulation. It is probable that the fetus absorbs the more saturated phospholipid fatty acids and that it has an especial avidity for the cephalin fraction of the phospholipids.

(4) The white blood cells of fetal blood contain about one half the lipid concentration of adult leukocytes which is probably due to the greater proportion of lymphocytes in the blood of the fe-

tus at birth. No evidence was obtained that they function in the transport of fat from the placenta to the fetus or vice versa.

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# VENOUS PRESSURE AND POSTURE IN NORMAL YOUNG WOMEN

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Much work in this laboratory has been concerned with circulatory phenomena in normal young women. Certain studies have given evidence of the very considerable variations shown in adjustments of heart rate and arterial pressures to changes of posture. These have been interpreted from the circulatory standpoint as, at one extreme, a picture of marked success and, at the other extreme, of marked failure in maintaining the erect posture. Since the controlled and adequate return of blood to the heart is a factor of major importance in such circulatory adjustments, measurements of venous pressures in hand and foot with the body in various positions seemed desirable especially when the erect posture is quietly maintained for a considerable period. The number of previous determinations bearing on this point is not large nor do the various observers altogether agree. Mention may be made of von Recklinghausen (1906), Hooker (1911), Barach and Marks (1913), and Carrier and Rehberg (1923). Eyster (1929, p. 33) referred to the work of Barach and Marks as authoritative. It seemed important, however, to study further in young women the varying circulatory success in postural adjustment. We were able to find only one subject with inferior power of adjustment available at this time, but since our basic results differ from those of others they are presented here.

The choice of method was important. It was clear that if determinations were to be repeated frequently during a period lasting often as long as two hours an indirect method was the only one possible. Also we have been skeptical about the use of the direct method of venous puncture for our group since the young college woman as we see her is far from phlegmatic. The possibility of changes in venous pressure due to alterations and fluctuations in muscular tension in a subject outwardly quiet seemed not improbable. Hooker (1911) called attention to the ease with which muscular activity alters venous relations. We also

have found that even slight muscular movements markedly alter the readings, for example, in the leg veins of a standing subject under observation by the indirect method.

The indirect method chosen was that of Krogh, Turner, and Landis (1932). The preliminary tests described in that paper were considered sufficient to establish the validity of the method. A few variations in procedure will be noted below. The method chosen is as free as possible from causes which might lead to alterations of pressure other than those due to postural changes and one in which ease of observation is maximal. The whole procedure involves careful attention to detail since the function is so labile. All measurements were made between November, 1932, and April, 1933. The subjects were healthy college students between the ages of 18 and 22 years. Each experiment required about two hours and all disturbing factors such as lack of sleep, minor infections, serious causes of worry, and especially interruptions to the routine of the experiment were avoided. The room temperature was maintained between 21° and 23° C., a temperature comfortable for the subject in her relaxed condition, also warm enough to favor easy observation of the skin veins. The aim was to secure a constant and normal flow of blood in the veins chosen. Preliminary work had shown that the uncovered hands and feet of a motionless subject tend to become cool even in a warm room, the amount of blood in the veins becomes markedly reduced and visualization of the flow becomes impossible just when accurate observation is essential. Washing the hands and feet in warm water before beginning the experiment and covering them with soft cotton pads except during the actual time of observation kept them constantly warm even though motionless and thus facilitated observation of the veins. Cross lighting was found best. A loose woolen bathrobe was the only clothing worn by the subject during the experiment, a procedure adopted to avoid all ex-

ternal pressure upon the venous route between the point of observation and the heart.

A typical experiment was conducted as follows. A celluloid capsule was cemented by collodion over a prominent vein on the back of the hand. A drying time of ten minutes was sufficient. It is necessary that the vein chosen for observation lie over a bone to insure firm support, otherwise the external counterpressure applied in the determination is partially dissipated in the soft tissues which leads to inaccurate readings. In the first series of observations the subject was in a horizontal position on a tilting table. The vein was brought approximately to the level of the right auricle and the hand was then carefully supported to insure steadiness. Pressure on the vein by the capsule itself was avoided by cutting notches in its celluloid margin to fit the special conformation of the vein chosen. A water manometer indicated the degree of pressure which was applied by a thick-walled rubber bulb controlled by a screw clamp. With a certain amount of pressure the vein was seen to collapse, indicating that the external pressure had become about high enough to overcome the internal pressure. Careful observation, however, showed that after this collapse a thin thread of blue was still discernible. A further increase of pressure was found to stop this slender stream in the mid-region of the field under observation. This interruption could be made to come and go by slightly changing the pressure through about 0.5–1.0 cm. water by moving the compressing screw or more readily by pinching the tube. The pressure not at the moment of collapse but at the point of definite interruption to the flow was taken as the true venous pressure. Several determinations were made, separated by a half minute or more. The period of collapse required for each observation was very short, not more than a few seconds for a trained observer.

While the subject was in a reclining position for the application of the capsule on the hand, another capsule was put over a vein in the foot, with the same precaution to secure a bony support. Two or three coats of collodion were found necessary to fasten this capsule firmly to the skin, because of the high pressure needed to collapse the foot veins when the subject was tilted to the erect position. A mercury manometer was substituted

for the water type employed for hand pressures; otherwise the apparatus for the veins of hand and foot was the same.

Determinations were made on the veins of both hand and foot at heart level with the subject in a horizontal position. This was always more than a half hour after the subject first reclined.

The subject was then tilted on the board to an angle of  $45^\circ$ , equivalent to a semi-erect position, with the hand kept at heart level, and after a period of fifteen minutes for circulatory adjustment to the new position a series of pressures in the veins of both hand and foot was recorded. The use of the tilting table avoided all muscular effort in changing position.

For a third series of observations the subject was tilted to an almost erect position, an angle of  $75^\circ$ . This angle has been found to be the steepest at which the subject remains passive and feels well supported by the tilting table. If a more erect position is taken the subject begins to feel that her support comes from her own feet, not from the table at her back, and her state is less steady and relaxed. In the  $75^\circ$  position the column of blood in the veins is influenced by gravity nearly as much as in standing, yet, as shown by metabolism experiments, Newton (1929), the muscular exertion necessary to maintain the erect position is almost entirely eliminated. The increase in oxygen consumption for this tilted position averaged only 5.6 per cent above that for reclining as against an increase of 18.5 per cent for quiet standing. Pressures in the veins of hand and foot were determined again after a fifteen minute period allowed for adjustment.

An important part of the apparatus used on the veins of the foot in the tilted positions was the calibrated counterweighting clamp devised by Krogh, Turner, and Landis. The use of this weight is not necessary on veins of hand or foot at heart level, but at levels considerably below that of the heart the higher pressures then required within the capsule induce cupping of the skin and hence faulty readings unless the counterweighting clamp is applied with sufficient force to keep the skin free from deformation. The calibration previously described was found less reliable for determining the necessary pressure on the clamp than a preliminary observation of the degree of counterweighting required. Skin defor-

# VENOUS PRESSURE AND POSTURE IN NORMAL YOUNG WOMEN

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Much work in this laboratory has been concerned with circulatory phenomena in normal young women. Certain studies have given evidence of the very considerable variations shown in adjustments of heart rate and arterial pressures to changes of posture. These have been interpreted from the circulatory standpoint as, at one extreme, a picture of marked success and, at the other extreme, of marked failure in maintaining the erect posture. Since the controlled and adequate return of blood to the heart is a factor of major importance in such circulatory adjustments, measurements of venous pressures in hand and foot with the body in various positions seemed desirable especially when the erect posture is quietly maintained for a considerable period. The number of previous determinations bearing on this point is not large nor do the various observers altogether agree. Mention may be made of von Recklinghausen (1906), Hooker (1911), Barach and Marks (1913), and Carrier and Rehberg (1923). Eyster (1929, p. 33) referred to the work of Barach and Marks as authoritative. It seemed important, however, to study further in young women the varying circulatory success in postural adjustment. We were able to find only one subject with inferior power of adjustment available at this time, but since our basic results differ from those of others they are presented here.

The choice of method was important. It was clear that if determinations were to be repeated frequently during a period lasting often as long as two hours an indirect method was the only one possible. Also we have been skeptical about the use of the direct method of venous puncture for our group since the young college woman as we see her is far from phlegmatic. The possibility of changes in venous pressure due to alterations and fluctuations in muscular tension in a subject outwardly quiet seemed not improbable. Hooker (1911) called attention to the ease with which muscular activity alters venous relations. We also

have found that even slight muscular movements markedly alter the readings, for example, in the leg veins of a standing subject under observation by the indirect method.

The indirect method chosen was that of Krogh, Turner, and Landis (1932). The preliminary tests described in that paper were considered sufficient to establish the validity of the method. A few variations in procedure will be noted below. The method chosen is as free as possible from causes which might lead to alterations of pressure other than those due to postural changes and one in which ease of observation is maximal. The whole procedure involves careful attention to detail since the function is so labile. All measurements were made between November, 1932, and April, 1933. The subjects were healthy college students between the ages of 18 and 22 years. Each experiment required about two hours and all disturbing factors such as lack of sleep, minor infections, serious causes of worry, and especially interruptions to the routine of the experiment were avoided. The room temperature was maintained between 21° and 23° C., a temperature comfortable for the subject in her relaxed condition, also warm enough to favor easy observation of the skin veins. The aim was to secure a constant and normal flow of blood in the veins chosen. Preliminary work had shown that the uncovered hands and feet of a motionless subject tend to become cool even in a warm room, the amount of blood in the veins becomes markedly reduced and visualization of the flow becomes impossible just when accurate observation is essential. Washing the hands and feet in warm water before beginning the experiment and covering them with soft cotton pads except during the actual time of observation kept them constantly warm even though motionless and thus facilitated observation of the veins. Cross lighting was found best. A loose woolen bathrobe was the only clothing worn by the subject during the experiment, a procedure adopted to avoid all ex-

ternal pressure upon the venous route between the point of observation and the heart.

A typical experiment was conducted as follows. A celluloid capsule was cemented by collodion over a prominent vein on the back of the hand. A drying time of ten minutes was sufficient. It is necessary that the vein chosen for observation lie over a bone to insure firm support, otherwise the external counterpressure applied in the determination is partially dissipated in the soft tissues which leads to inaccurate readings. In the first series of observations the subject was in a horizontal position on a tilting table. The vein was brought approximately to the level of the right auricle and the hand was then carefully supported to insure steadiness. Pressure on the vein by the capsule itself was avoided by cutting notches in its celluloid margin to fit the special conformation of the vein chosen. A water manometer indicated the degree of pressure which was applied by a thick-walled rubber bulb controlled by a screw clamp. With a certain amount of pressure the vein was seen to collapse, indicating that the external pressure had become about high enough to overcome the internal pressure. Careful observation, however, showed that after this collapse a thin thread of blue was still discernible. A further increase of pressure was found to stop this slender stream in the mid-region of the field under observation. This interruption could be made to come and go by slightly changing the pressure through about 0.5–1.0 cm. water by moving the compressing screw or more readily by pinching the tube. The pressure not at the moment of collapse but at the point of definite interruption to the flow was taken as the true venous pressure. Several determinations were made, separated by a half minute or more. The period of collapse required for each observation was very short, not more than a few seconds for a trained observer.

While the subject was in a reclining position for the application of the capsule on the hand, another capsule was put over a vein in the foot, with the same precaution to secure a bony support. Two or three coats of collodion were found necessary to fasten this capsule firmly to the skin, because of the high pressure needed to collapse the foot veins when the subject was tilted to the erect position. A mercury manometer was substituted

for the water type employed for hand pressures; otherwise the apparatus for the veins of hand and foot was the same.

Determinations were made on the veins of both hand and foot at heart level with the subject in a horizontal position. This was always more than a half hour after the subject first reclined.

The subject was then tilted on the board to an angle of  $45^\circ$ , equivalent to a semi-erect position, with the hand kept at heart level, and after a period of fifteen minutes for circulatory adjustment to the new position a series of pressures in the veins of both hand and foot was recorded. The use of the tilting table avoided all muscular effort in changing position.

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mation varies with the elasticity and tightness of the skin in different subjects and in different positions on the foot. It is also obvious that where venous pressures are high and the veins distended the notches in the rim of the capsule must be cut deep enough to avoid any lowering of the measurement due to cutting off a portion of the venous pressure by the rim of the capsule, especially when counterweighted. It is suggested that certain of the widely varying results obtained by those using the capsule method may be due to the intrinsic difficulties of applying an unnotched capsule.

The observations were recorded in the usual type of protocol. The data were then treated as in Table I, which gives two typical experiments for Subject G. Foot pressures originally taken

TABLE I

*Venous pressures observed in two experiments on Subject G. To show changes in pressure with tilting to 45° and 75°. Hand vein kept at heart level. Hydrostatic factor shows vertical distance between right auricle and foot vein. Experiments indicate degree of uniformity obtained in readings.*

Vein used	Horizontal	45° angle	75° angle	Hydrostatic factor
	cm. H <sub>2</sub> O	cm. H <sub>2</sub> O	cm. H <sub>2</sub> O	cm. H <sub>2</sub> O
Experiment I, January 21, 11:00 a.m.				
Hand vein	7.4 6.8 7.0	6.8 7.5 7.2	7.2 6.8 6.7 6.5	
Foot vein	10.1 10.5 10.1	81.0 83.7 86.4	110.7 113.4 114.7 114.7	80.5 (45°) 110.0 (75°)
Experiment II, February 23, 11:00 a.m.				
Hand vein	7.0 6.5 7.0	6.0 7.5 7.0	6.8 7.2 7.2	
Foot vein	10.1 10.5 11.0	85.0 83.7 87.7	112.0 114.7 118.8 118.8	80.5 (45°) 110.0 (75°)

in mm. Hg are converted into cm. water for convenience of comparison. The table includes, in the column headed, "Hydrostatic factor," the figures for the influence of gravity on the circulation in the tilted positions, obtained by measuring the

distance in cm. between the foot vein and the level of the right auricle. Similar data for Subject A are shown graphically in Figure 1 where the ordi-

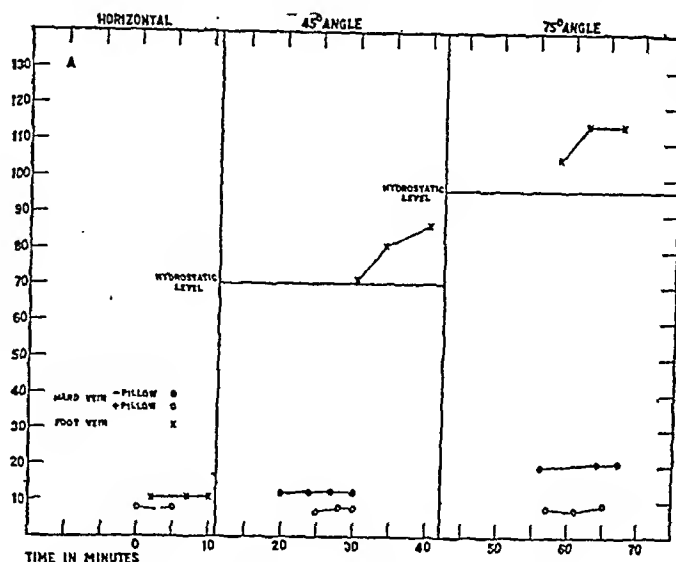


FIG. 1. VENOUS PRESSURES OBSERVED IN SUBJECT A.

Ordinates, venous pressures in cm. water. Abscissae, time in minutes beginning with the first determination. Preliminary rest period of about 40 minutes not included.

Two experiments are shown. One, the effect of tilting on pressures in veins of hand and foot, shown by open circles and crosses respectively. The other, the effect of a small pillow under the shoulder on pressure in veins of hand at heart level. Results identical in horizontal position, shown by open circles. For tilted positions: open circles, with pillow; solid dots, without pillow.

nates represent centimeters of water, the abscissae time in minutes. The circles show observations on the vein of the hand, the crosses those on the vein of the foot. The hydrostatic factors are shown for the tilted positions by horizontal lines.

These tables and graphs, which are very similar for the seven subjects, demonstrate that the venous pressure in the large superficial veins of the hands was quite constant in a given subject during the experimental period and with varying posture. This constancy was noted in each of the fourteen experiments chosen as entirely above reproach. Sixteen completed experiments were omitted because of the obvious effect of slight interruptions, delays due to technical difficulties and the like. This constancy of venous pressure in the hand with variations in posture does not support the results of Barach and Marks (1913) who studied arterial and venous pressure in twenty-six human subjects placed in erect and horizontal positions

on a tilting table. They reported a rise of venous pressure in a vein of the arm when their subjects assumed the erect position, amounting in some cases to as much as 18 cm. water. In connection with their results it is significant that in a series of experiments preliminary to those we have described we also observed an increase of venous pressure when the subject was tilted from horizontal to a 45° angle and a further increase on tilting to 75°. With the insertion, however, of a small pillow under the shoulder, a manoeuvre first adopted for the comfort of the subject, this increase completely disappeared. This increase observed by others, and also at first by ourselves, may arise from the fact that as the subject lying on a flat table is tilted to more erect positions, the line of the clavicle may be altered by the downward pull of the weight of the arm thus lessening

TABLE II

*Venous pressures observed in two experiments on Subject C. To show change induced by placing a small pillow under shoulder.*

Condition	Horizontal <i>cm. H<sub>2</sub>O</i>	45° angle <i>cm. H<sub>2</sub>O</i>	75° angle <i>cm. H<sub>2</sub>O</i>
Experiment I			
Hand vein with pillow under shoulder	7.0	6.5	5.0
	6.3	7.2	7.0
	6.2	7.0	6.5
Hand vein without pillow under shoulder	7.0	10.0	18.2
	6.8	9.2	18.7
	6.5	11.5	19.0
	6.5	12.0	19.2
Experiment II			
Hand vein with pillow under shoulder	6.9	7.0	6.9
	6.5	6.8	7.0
	6.8	6.8	7.0 6.2
Hand vein without pillow under shoulder	7.2	10.5	18.5
	7.0	10.7	18.6
	6.6	10.7	18.8
	6.5		18.8

the space through which the subclavian vein passes between clavicle and first rib. Such a compression of the vein might result in the misleading increase in venous pressure observed in the hand. All experiments were carried on with a small pillow under the shoulder except when

this phenomenon was being studied. Table II shows in two experiments for Subject C the characteristic results for pressures in the vein of the hand, with and without the pillow. Similar results are also included in Figure 1 by the black dots for results without the pillow in the tilted positions. The results for the horizontal position are approximately the same with and without the pillow, therefore the small circles satisfy both conditions.

In all experiments in the horizontal position the pressure in the vein of the foot was higher than in the vein of the hand though both were at the level of the heart. The veins of the foot are farther from the heart. Previous work in this laboratory, unpublished, has shown the arterial pressure in the leg to be somewhat higher than that in the arm at heart level; therefore it is not improbable that this higher pressure at the greater distance is required to maintain the gradient of the circulation.

Determinations on veins of the foot were consistent in showing a progressive rise in pressure when the subject was tilted to 45° and further to 75°. In each position the pressure was practically constant in a given individual after the initial period of fifteen minutes for adjustment. In every subject the venous pressure was somewhat higher than the hydrostatic counter pressure calculated from the difference of levels between the vein observed and the heart. If the blood is to return steadily to the heart in a quiet subject, it is obvious that the venous pressure must at least equal the hydrostatic factor unless the return is dependent upon respiratory or other aids. In Subject A, for example, at an angle of 45° the hydrostatic factor was approximately 70.7 cm. water; the average of three determinations of venous pressure in the vein of the foot was 79.5 cm. When tilted to 75° the hydrostatic factor was 96.5 cm., the average venous pressure was 111.5 cm. The effect of posture was thus pronounced, and in both tilted positions venous pressure was ample to effect the return of blood to the heart. Such successful adjustments were observed in each of the two experiments on all seven subjects.

These results do not agree with those previously reported by von Recklinghausen (1906), by Hooker (1911), and by Carrier and Rehberg



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	cm. H <sub>2</sub> O	cm. H <sub>2</sub> O	cm. H <sub>2</sub> O	cm. H <sub>2</sub> O

Experiment I, January 21, 11:00 a.m.

Hand vein	7.4	6.8	7.2	
	6.8	7.5	6.8	
	7.0	7.2	6.7	
Foot vein	10.1	81.0	110.7	80.5 (45°) 110.0 (75°)
	10.5	83.7	113.4	
	10.1	86.4	114.7	

Experiment II, February 23, 11:00 a.m.

Hand vein	7.0	6.0	6.8	
	6.5	7.5	7.2	
	7.0	7.0	7.2	
Foot vein	10.1	85.0	112.0	80.5 (45°) 110.0 (75°)
	10.5	83.7	114.7	
	11.0	87.7	118.8	

in mm. Hg are converted into cm. water for convenience of comparison. The table includes, in the column headed, "Hydrostatic factor," the figures for the influence of gravity on the circulation in the tilted positions, obtained by measuring the

distance in cm. between the foot vein and the level of the right auricle. Similar data for Subject A are shown graphically in Figure 1 where the ordi-

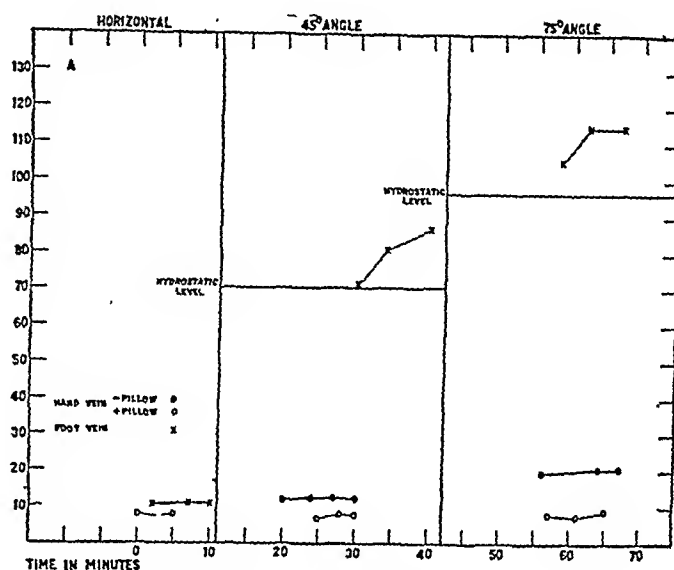


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TABLE II

*Venous pressures observed in two experiments on Subject C. To show change induced by placing a small pillow under shoulder.*

Condition	Horizontal cm. H <sub>2</sub> O	45° angle cm. H <sub>2</sub> O	75° angle cm. H <sub>2</sub> O
Experiment I			
Hand vein with pillow under shoulder	7.0	6.5	5.0
	6.3	7.2	7.0
	6.2	7.0	6.5
Hand vein without pillow under shoulder	7.0	10.0	18.2
	6.8	9.2	18.7
	6.5	11.5	19.0
	6.5	12.0	19.2
Experiment II			
Hand vein with pillow under shoulder	6.9	7.0	6.9
	6.5	6.8	7.0
	6.8	6.8	7.0 6.2
Hand vein without pillow under shoulder	7.2	10.5	18.5
	7.0	10.7	18.6
	6.6	10.7	18.8
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the space through which the subclavian vein passes between clavicle and first rib. Such a compression of the vein might result in the misleading increase in venous pressure observed in the hand. All experiments were carried on with a small pillow under the shoulder except when

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These results do not agree with those previously reported by von Recklinghausen (1906), by Hooker (1911), and by Carrier and Rehberg



(1923). In the veins of the foot in sitting and standing positions von Recklinghausen found that the rise of venous pressure was not sufficient to overcome the opposing hydrostatic factor although it was sufficient to bring the blood to about the entrance to the body cavity. Discrepancies of somewhat lesser magnitudes were found by the other workers. Hooker noted that the quieter the subject the higher the venous pressure in the standing position and that the pressure in a vein of the foot with the subject reclining in a steamer chair might be greater than the hydrostatic factor. Carrier and Rehberg noted that continued maintenance of the standing position caused the venous pressure to approach the values for the hydrostatic factor though always lower. In their work the subject stood on one foot on a stool with the experimental leg hanging relaxed, but fatigue limited the duration of possible observation. If the subject stood on both feet, thus involving muscular effort, the venous pressures in the lower veins were about 20 cm. less. None of these methods made use of a tilting table and it is possible that in spite of intentions to the contrary the blood was helped along by the contraction of the muscles of the leg. The capsules in some studies were not of large size nor were they counterweighted. It is important to note that in our experiments the subjects were entirely relaxed and quiet for a considerable time before as well as during the period of observation, and since these young women felt no trace of dizziness while tilted their circulatory state presumably was reasonably steady.

Though the series is not large enough for statistical treatment, Table III shows the total ranges of venous pressures obtained and the averages for the different positions. The ranges are not wide in comparison with some found in the literature. Some of the very high values reported by others may have been due to a lack of bony support for the vein chosen and a consequent dissipation in the soft tissues of the pressure applied. Also for high pressures the cupping of the skin under the capsule leads to incorrect readings, which were prevented in our work by the counterweighting clamp. There may be a variation in the success with which the end-point is observed for some capsules leave a very limited portion of the vein visible and that none too clearly.

TABLE III

*Determinations of venous pressure.* Subjects, seven healthy young college women. Indirect method. In cm. water.

Postural condition	Average	Total range	Range of 75 per cent of readings
<i>Hand veins at heart level</i>			
Horizontal.....	7.2	6.0- 8.4	6.6- 8.0
Tilted to 45°.....	7.9	6.0- 9.5	7.0- 8.7
Tilted to 75°.....	7.0	5.0- 9.0	6.5- 8.0
<i>Foot veins</i>			
Horizontal.....	10.5	6.4- 12.8	8.9- 11.0
Tilted to 45°.....	78.4	70.2- 87.7	75.0- 85.0
Tilted to 75°.....	114.4	102.6-124.2	109.3-120.0

Eyster gives 4 to 6 cm. water as the normal range of venous pressure in the superficial veins of the hand at heart level. Our range is slightly higher, 6 to 8 cm. This difference may easily be due to a clearer field of observation or to the use of a slightly different end-point, the actual interruption of the flow in the vein rather than the first collapse.

*Note on pressure in a subject with inferior circulatory adjustments*

While the seven subjects discussed above all showed successful adjustments to the erect posi-

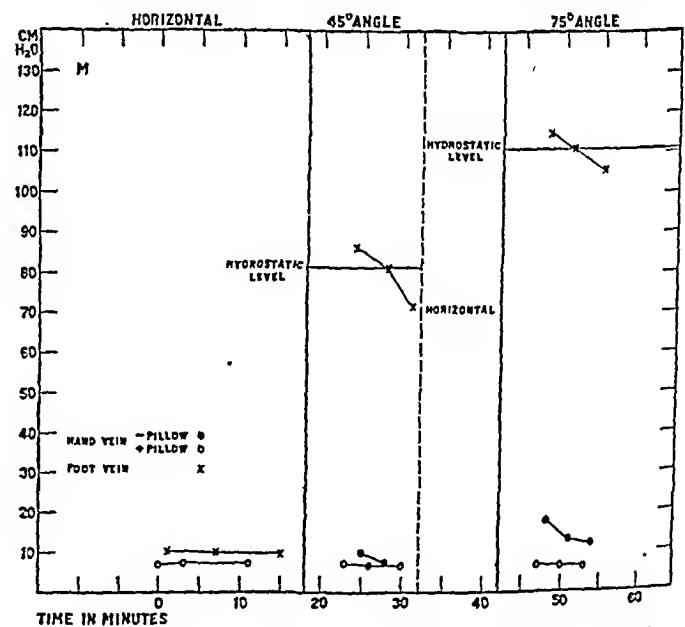


FIG. 2. SUBJECT M, OF INFERIOR CIRCULATORY ADJUSTMENT, UNABLE BECAUSE OF FAINTNESS TO ENDURE TILTED POSITIONS LONGER THAN INDICATED BY TIME OF DETERMINATIONS.

Symbols as in Fig. 1.

tion during the half hour study, an eighth subject repeatedly became dizzy and fainted after six to eight minutes at 75° or even at 45°. From Figure 2 it is readily seen that the pressures in the vein of her foot when she was tilted to 45° and 75° only once equalled the hydrostatic factor, and also that a steady fall in pressure in her foot occurred until dizziness made the erect or semi-erect position untenable. A slight, possibly not significant, fall is seen in the pressure in her hand at heart level at the same time. Turner, Newton, and Haynes (1930) showed that quiet, healthy, standing young women make extensive circulatory adjustments which are maintained as the quiet position is prolonged although marked variations occurred in different individuals. They observed a distinct and progressive increase in the heart rate, a rise in diastolic pressure, and a lessened pulse pressure when subjects were tilted progressively through a series of angles up to 90°, with all these changes much more marked in subjects who habitually became dizzy when erect and still. There was also evidence of increased volume in the legs due to stagnation of fluid during quiet standing, and evidence for stagnation of blood in the abdominal region. The measurements of venous pressure in our one subject with inferior circulatory adjustment seem to extend the data just summarized for such persons. The low venous pressure in her foot may indicate a failure in the return of blood to the heart. The color of her feet, much more purple than those of the other subjects, also suggested this. It is to be regretted that no other subjects of this type were available.

#### SUMMARY

1. Determinations of venous pressure by the indirect method of Krogh, Turner, and Landis have been made on the superficial veins in the hands and feet of seven normal healthy college women while they were subjected to postural changes on a tilting board. The horizontal position and tilted positions at angles of 45° and 75° were used.

2. Venous pressure in the superficial veins of the hand at heart level following a preliminary pe-

riod for adjustment remained constant for each individual for periods of over an hour, in spite of changes in posture. The effect of the position of the shoulder on the venous pressure in the hand is discussed. The average pressure in the veins of the hand was 7.2 cm. water.

3. The venous pressure in the superficial veins of the foot at heart level, 10.5 cm. water, was somewhat higher than that of the hand in the reclining position.

4. Venous pressure in the veins of the foot increased with change in posture from the horizontal to 45° and 75° angles; for each position it remained approximately constant during the experimental period; at both 45° and 75° it was somewhat greater than the hydrostatic factor. In healthy subjects who experienced no dizziness in the tilted positions the return of blood therefore seemed assured by venous pressure alone.

5. Readings of venous pressure in one subject with inferior circulatory adjustment when tilted to 75° showed a pressure in the vein of the foot inadequate to overcome the hydrostatic factor.

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# A SURGICAL TREATMENT OF ESSENTIAL HYPERTENSION

BY IRVINE H. PAGE AND GEORGE J. HEUER

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(Received for publication August 3, 1934)

Evidence of varied nature indicates that the arterioles of the splanchnic region are constricted in patients suffering from essential hypertension, and that this may be an important factor in maintaining the pressure at an elevated level. Interruption, by surgical means, of nerves carrying effector impulses to this region would therefore appear to be a logical therapeutic procedure.

Section of the anterior nerve roots close to the cord seems a method which will surely intercept both the motor and sympathetic innervation. It is furthermore desirable that denervation be as complete as possible without interfering with vital functions. Anatomical considerations suggest that this can best be accomplished by section of the anterior roots from the sixth thoracic segment to, and including, the second lumbar.

The sixth to the twelfth thoracic motor nerves innervate the intercostal muscles and the seventh to first lumbar nerves supply the abdominal musculature. Part of the supply to the flexor muscles of the hip is derived from the first and second lumbar nerves. The first lumbar root contributes branches to the ileoinguinal and ileohypogastric nerves; and both the first and second roots send fibers to the genito-femoral. The lateral cutaneous nerves receive fibers from the first, second and third lumbar roots.

There is some difference of opinion as to the anatomy of the sympathetic system in the thoraco-lumbar region. All of the anterior nerve roots contribute to the innervation of the cutaneous vessels, pilomotor muscles and sweat glands. The major splanchnic nerves are derived from the fifth to the tenth thoracic segment, and the minor splanchnic from the ninth and tenth or the tenth to twelfth roots. These nerves join the celiac plexus. In this manner connection is established with the stomach, liver, adrenals, pancreas, intestine and kidneys. Section of the sixth thoracic to the second lumbar roots, therefore, partially interrupts not only the motor nerve supply of the ab-

dominal wall but the sympathetic supply of most of the abdominal viscera, except the colon, rectum, bladder and genital organs. The vagus and phrenic nerves and the intrinsic nerve supply remain intact to innervate the viscera. The inferior mesenteric ganglion also remains intact except for loss of some fibers from the second lumbar root.

Adson and Brown (1) (1934) were the first to suggest and perform the operation as outlined. By this procedure they hoped (1) to remove the sympathetic innervation of sufficient arteries to modify arterial responses, (2) thoroughly to denervate the suprarenal glands, and (3) to remove the effects of intra-abdominal tension. They report a case of early malignant hypertension in a woman 29 years of age who had had high blood pressure for at least 18 months. The blood pressure in the recumbent position varied from 150 to 200 systolic and from 100 to 150 diastolic. The range following operation was from 155 to 195 systolic and from 100 to 150 diastolic. After operation no visible sweating was found below the level of the epigastric notch anteriorly or below the angle of the scapulae posteriorly. There was no difference in the amount of phenolsulphonphthalein excreted whether the patient was standing or recumbent. Evidently considerable sclerosis of the vessels was present in this case. After operation the retinal vessels remained narrowed. Retinitis was, however, less active than on admission. The response to Brown's test of pressor response to cold showed that following operation there was a significant decrease in the maximum rise in blood pressure. While the operation did not lower the systemic blood pressure level as much as might have been hoped for, it was believed that this was due to structural changes in the blood vessels.

It therefore seemed to us desirable to select a case in which the vascular tree was still flexible and yet one in which the hypertension was a potential source of grave danger to life.

*Hospital No. 8990.* The patient, a 23 year old Jewish girl, complained of fatigue and restlessness for a period of at least one year and a half. She had had good health up to the time of her present illness. Temperamentally, however, she was excessively excitable and unstable. Her friends found her a "difficult" person.

A chance physical examination showed that her blood pressure was over 200 mm. Hg. Until a few months ago she was symptom-free except for the feeling of excessive fatigue. Headaches were frequent and she suffered from palpitation and dull pains over the precordium. At night her ankles swelled.

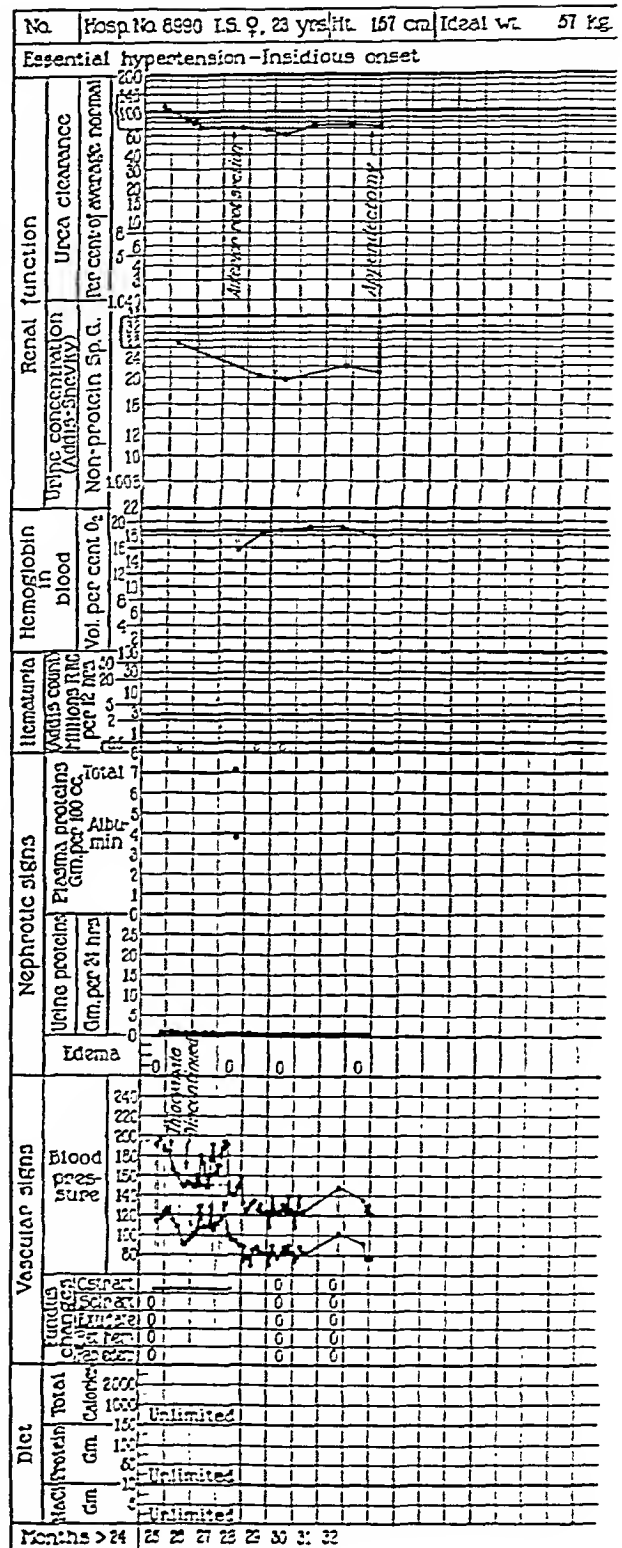
The patient was a well developed, highly nervous girl. She had advanced acne vulgaris over her face, chest, and back. The hair distribution was normal. The fundus examination demonstrated that the nerve head was hyperemic, the arterioles were slightly constricted and the veins dilated. There were no retinal hemorrhages or exudates. The tonsils were buried and ragged and the thyroid gland was not enlarged. The heart measurements were as follows: M.L. = 8.3 cm., M.R. = 4.6 cm. The chest diameter was 23 cm. The cardiac sounds were of good quality and A<sub>2</sub> and P<sub>2</sub> were slightly accentuated. The peripheral vessels were not tortuous or thickened. The blood pressure was 200/140 mm. Hg. The kidneys were palpable but not enlarged. The superficial and deep reflexes were hyperactive. The hands and feet were cold and perspired freely.

This girl seemed to be suffering from essential hypertension of relatively short duration. Her temperament undoubtedly greatly intensified the disease as we had occasion to observe on many occasions. Her vascular system was still flexible as evidenced by the facts that amyl nitrate, mecholol (acetyl  $\beta$ -methyl cholin) and postural changes produced marked diminution in her blood pressure. There was also neither palpable sclerosis of her peripheral vessels nor visible changes, except constriction in her retinal arterioles. Cardiac decompensation was evident under strain, for edema of her ankles appeared only after a day's work.

The patient was confined to bed in the hospital and the level of the blood pressure measured at 9:30 every morning. Ten days after admission sodium thiocyanate was administered over a period of 20 days. It was necessary also to give chloral hydrate (0.5 gram t.i.d.) and amylal at night. A graphic chart (Figure 1) has been prepared to show the course of the patient while in the hospital.

Thiocyanate treatment apparently caused the level of the blood pressure to fall markedly but, on discontinuing the drug, it rose to its original level. After about ten weeks of strict bed rest it was evident that the blood pressure would not fall more than it had. It varied from a maximum of 192/134 to 162/108 mm. Hg. Since the patient showed no improvement it was our belief that radical surgical intervention was justifiable.

*Surgical procedure.* The operation was performed May 11, 1934, under ether anesthesia. It consisted of the exposure and removal of the laminae corresponding with the sixth thoracic to the second lumbar segments



inclusive; and the incision of the dura mater so as to expose the spinal cord. With an appropriate instrument each pair of anterior nerve roots was separated from the spinal cord as it coursed along this structure and divided approximately midway between its point of origin and its union with the posterior roots. Silver clips were placed across several roots, the division of which was associated with slight hemorrhage. After completing the division of the roots the wound was closed throughout with silk. The operation required three hours. The pulse during operation varied between 110 and 90.

Recovery from the operation was uneventful. The blood pressure fell rapidly and progressively to a level that was nearly normal. Especially striking was the fall in diastolic pressure. For the first week after operation the patient had moderate difficulty in evacuating her bowels. This was remedied by use of enemata. The abdominal muscles were completely paralyzed. Although relaxed, when the patient stood up, there was almost no "pot belly." An abdominal binder was not found necessary. She soon learned how to manage the motion of her trunk without the use of the abdominal musculature.

There was steady improvement in the patient's strength but her emotional volatility continued unabated. Her headaches, palpitations and precordial pain, however, disappeared.

#### *Laboratory examinations before and after operation*

The renal function, as measured by the urea clearance test, was found to be normal on six

occasions before operation. Nor did it change significantly following operation. The ability of the kidneys to concentrate was also not markedly impaired though the maximum non-protein specific gravity<sup>1</sup> fell from 1.027 to 1.019, measurements being made on a 12-hour specimen voided at the end of 24 hours without fluids. Neither before nor after operation was there an increase in the number of red blood cells or casts counted in Addis sediment test specimens of urine. Sugar was not found in the urine.

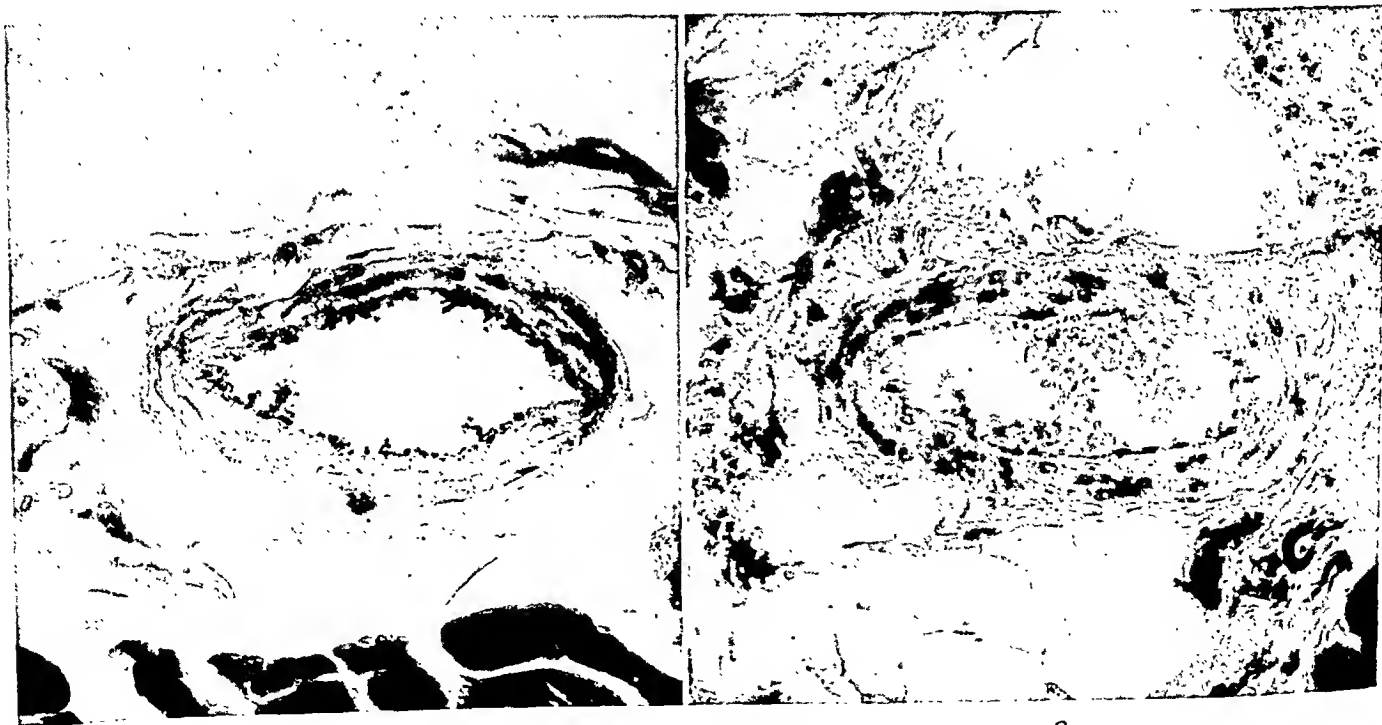
The plasma proteins were normal as was the hemoglobin. The latter fell slightly following operation but quickly returned to normal.

The basal metabolic rate was normal both before and after operation.

An electrocardiogram taken about two months before operation showed the following characteristics: T waves + + +, conduction time 0.17 seconds, R<sub>s</sub> split, low voltage and normal rhythm. Another record taken two months after operation showed: T waves + + —, conduction time 0.16, R<sub>s</sub> split and normal rhythm.

A muscle biopsy removed from the lumbar

<sup>1</sup> Obtained by subtracting 0.003 from the total specific gravity for each one per cent of protein (6).



1.

SECTION OF ARTERY OF NORMAL MAN 40 YRS. OLD.  
× 450.

2.

SECTION OF PATIENT'S ARTERY.  
× 500.

FIG. 2.

muscle during operation showed, according to Dr. C. P. Rhoads, that moderate but definite intimal thickening had taken place in the arterioles. (See Figure 2.)

Dr. William Lewis measured the minute volume output of this patient 26 days after operation by the acetylene procedure (Grollman et al. (2)). The following results were obtained:

Cardiac output, liters per minute = 4.1.

Arteriovenous oxygen difference per liter blood = 53.4 cc.

Cardiac output, liters per minute per square meter of body surface = 2.9.

Basal metabolic rate = + 7 per cent (Dubois standard).

The level of minute volume output is higher than the average normal ( $2.2 \pm 10$  per cent). Since only one determination was made it should not be considered as constantly elevated. It seems certain at least that it is not reduced below normal level as the result of operation.

The content of lipid in the plasma was ascertained before and after operation by the method of Kirk, Page and Van Slyke (1934).

level by anterior nerve root section. The blood pressure has remained at a level which is almost normal for seven months, in spite of the fact that the patient was allowed to go about the hospital wards without restriction. Emotional excitement caused the pressure to rise but seldom did it exceed 160 mm. Hg. Nor did it remain persistently elevated but rather quickly fell to its original level.

The ability of the kidneys to excrete urea, as measured by the urea clearance test, was unaltered as the result of almost complete denervation resulting from rhizotomy. It is interesting to note that no change in efficiency of the kidneys resulted even though a marked fall in blood pressure occurred. This corroborates the observation of Page (1934) that the efficiency of the kidneys in cases of essential hypertension and nephritis is not directly dependent on the level of systemic blood pressure. The power of the kidneys to concentrate urine was somewhat reduced.

Hemoglobin, plasma proteins, urine proteins, urine sugar and plasma lipids were not appreciably altered following operation. The basal metabolic rate and cardiac output both remained

TABLE I  
*Lipid content of plasma during fasting*

Date	Total lipid carbon	Total cholesterol	Free cholesterol	Lipid amino nitrogen	Lipid nitrogen	Lipid phosphorus	Total phosphatide†	"Cephalin"‡
	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.
March 6, before operation.....	479	192	72.6	2.22	6.14	5.47	129	118
June 22, after operation.....	528	241	88.1	6.33	20.4	8.65	202	235
Normal average values for young men	528	212	83	3.1	9.7	6.3	147	174

† Total phosphatide calculated from lipid phosphorus.

‡ "Cephalin" calculated from lipid amino nitrogen.

According to the values found by Page, Kirk and Van Slyke (1935) to be normal for young men, these results may be considered to fall within normal limits. The lipid amino nitrogen or "cephalin" value found after operation is slightly higher than normal.

#### DISCUSSION

Although the level of arterial blood pressure in this patient was high and tended to remain so, nevertheless the vascular system was flexible. It is our belief that as a consequence of this flexibility it was possible to reduce the blood pressure

normal. There was no increase above normal of the excretion of red blood cells in the urine.

#### CONCLUSIONS

1. Section of the anterior nerve roots from the 6th thoracic to the 2d lumbar segment has been performed in a young girl suffering from persistently elevated arterial blood pressure. Anatomical and physiological evidence indicated that her vascular system was yet flexible.

2. The blood pressure level quickly fell to normal, and has remained normal for seven months.

3. Denervation of the kidneys resulting from

the operation did not alter their power to excrete urea but there was slight loss in ability to concentrate urine. The kidneys were as efficient when the blood pressure was reduced to normal as when it had been elevated. No increase occurred in the number of red blood cells excreted in the urine.

4. In spite of the fact that the blood pressure fell to normal, there was no change in the subjective feeling of the patient except that headaches, palpitation, and precordial pain disappeared.

5. No significant alterations occurred in the hemoglobin, and plasma proteins. The basal metabolism and cardiac output also were normal. Electrocardiographic records taken before and after operation showed no change from normal.

6. Determinations were made of total lipid, total and free cholesterol, lipid amino nitrogen, total lipid nitrogen and lipid phosphorus in the blood plasma both before operation, when the blood pressure was elevated, and after when it was normal. Except for slight rise in lipid amino nitro-

gen and total lipid nitrogen, following operation, the changes were insignificant.

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# THE EFFECT OF RENAL DENERVATION ON THE LEVEL OF ARTERIAL BLOOD PRESSURE AND RENAL FUNCTION IN ESSENTIAL HYPERTENSION

By IRVINE H. PAGE AND GEORGE J. HEUER

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the Department of Surgery, New York Hospital, New York)

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Hypertension is one of the most frequent complications of renal disease, both acute and chronic. The fact that in nephritis renal influences, nervous or other, apparently produce hypertension suggests the possibility that the unknown cause of essential hypertension may lie in nervous impulses from the kidney, even though the kidneys themselves appear to be entirely normal at the onset of the hypertension.

We have accordingly performed bilateral renal denervation on a patient with essential hypertension uncomplicated by detectable renal involvement, and with but slight evidence of anatomical changes in the circulatory system. Because the hypertension was still in its initial stage, without organic damage, it appeared possible that therapeutic lowering of the blood pressure might occur.

## History of the patient

This 25-year old girl complained that she became easily fatigued and was extremely nervous. Cardiac disease had been found in her father and two sisters. At the age of 13 she had chorea with involvement of her heart. Six years later a tonsillectomy was performed because of swelling and pain in certain of her joints. Low grade hypertension was found during an examination before the operation. Since then she had not been troubled by symptoms of rheumatic fever, but hypertension had continued and became pro-

gressively more marked. In the past few years she had noticed that she blushed spontaneously, and that she cried for no apparent reason. She also had palpitations, her mouth became dry and she often had attacks of trembling. During the past three years, about two or three times a year, she had had mild fits suggestive of epilepsy. Until recently she had had many severe headaches, especially pronounced a few days before her menstrual period.

*Physical examination.* The patient was a well developed but thin girl with a markedly masculine build and hair distribution. There was a pronounced blotchy blush over the face, neck and upper thorax. Her hands and feet were cold and moist with perspiration.

Examination of the fundus revealed that the nerve head was of a deep pinkish color without papilledema. The arterioles were slightly constricted and definitely tortuous. Perivasculitis was not evident. The veins were tortuous and dilated and arteriovenous constriction was seen. There was no exudate or hemorrhage in the retina. Vision was good in all sectors.

There was marked systolic pulsation in the jugular notch. The thyroid gland was normal to palpation. The beating of the heart caused a marked thrust and the rate was rapid but regular. The heart appeared very slightly enlarged to the left. The first sound at the apex was snapping in

TABLE I  
*Lipid of plasma from fasting patient*

	Total lipid carbon	Cholesterol			Lipid amino nitrogen	Total lipid nitrogen	Lipid phos- phorus	Total fat	"Cephalin" calculated from NH <sub>3</sub> nitrogen	Total phosphatide calculated from phosphorus
		Total	Free	Ester						
	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.
Patient.....	439	241	77	164	2.5	8.9	5.7	570	132	134
Average normal	528	212	83	129	3.1	9.7	6.3	664	174	147



character associated with a soft presystolic murmur and a short diastolic whiff.  $A_2$  and  $P_2$  were slightly accentuated. The peripheral blood vessels were moderately thickened but not tortuous. The blood pressure was 208/140 mm. Hg.

The abdomen was scaphoid. Both kidneys were palpable but not enlarged. Tremor was not observed in the fingers when the arms were outstretched. The superficial and deep reflexes were equal on both sides and hyperactive. There was a slight bilateral ankle clonus.

**Laboratory examination.** Renal function, as measured by the urea clearance test of Møller, McIntosh and Van Slyke (1), was 91.5 per cent of average normal. The Addis sediment test showed that no significant number of red blood cells or casts were being excreted in the urine, that the specific gravity was 1.030, and that the protein excreted was not greater than 0.02 gram in 12 hours. Sugar was not found in the urine. The basal metabolic rate was  $+5$ . The hemoglobin was 20.5 volumes per cent oxygen capacity. The white blood cells numbered 8000 and the Kline test was negative.

Estimation of the plasma lipids by the method of Kirk, Page, and Van Slyke (2) showed that they were all within normal limits. (For normal values see Page, Kirk, and Van Slyke (3).)

X-ray examination of the heart showed that it was but little enlarged and of normal shape. The measurements were as follows: M.L. = 8.7 cm. M.R. = 3.9 cm. Internal diameter of thoracic cage = 22 cm. The peripheral vessels showed no calcification and the sella turcica was normal. The electrocardiogram exhibited the following characteristics: T waves  $+++$ ; conduction time 0.16;  $P_1$  and  $P_2$  split; normal rhythm.

**Course.** For convenience the laboratory and certain of the clinical observations are plotted in Figure 1. The points on the blood pressure record represent average blood pressure levels.

The blood pressure, taken daily at 9:30 a.m. with the patient strictly confined to bed at all times, showed little tendency to fall. Nor did a course of sodium thiocyanate therapy (up to grains iv O.D.) have any marked effect.

The kidneys were entirely normal in so far as could be ascertained by clinical examination. Renal function was normal as measured by the power of the kidneys to remove urea from the

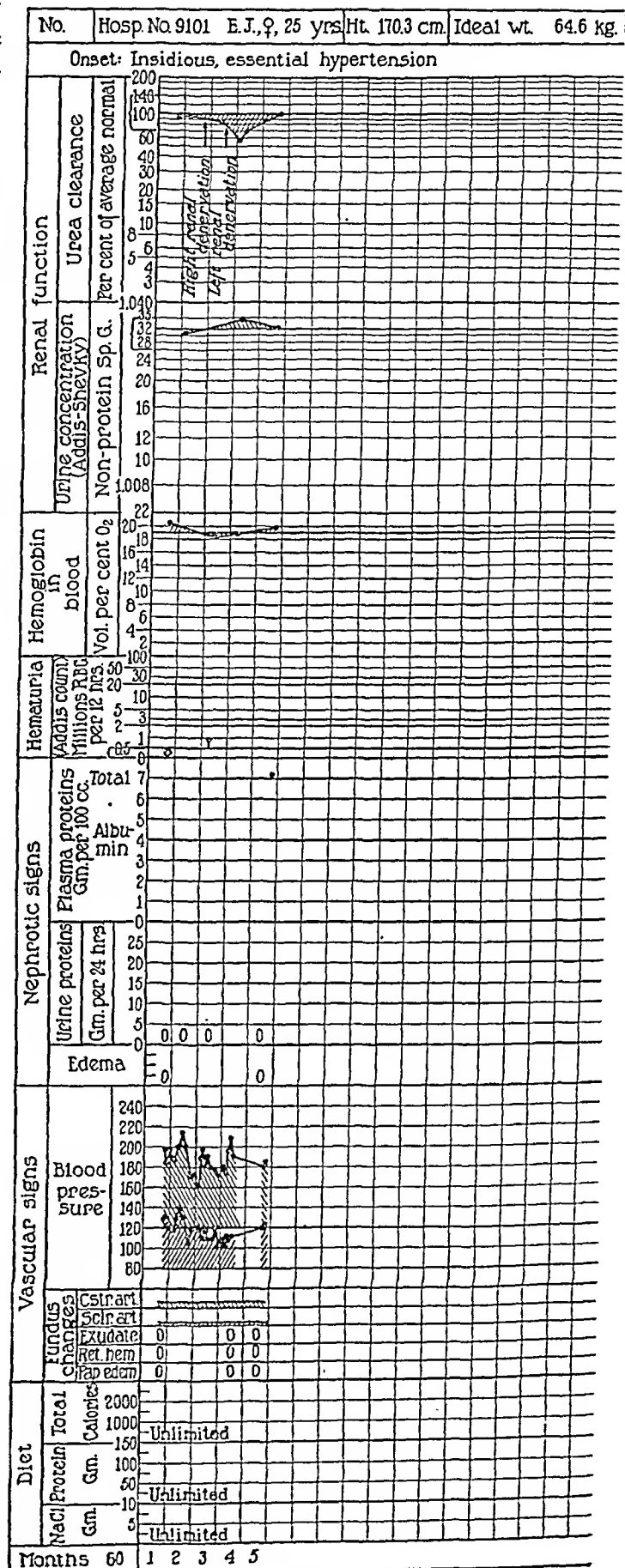


FIG. 1.

blood and by ability to concentrate the urine. No appreciable quantity of protein or red blood cells was excreted into the urine, nor was anemia present.

About one month after admission, since the patient did not appear to be improving, denervation of the right kidney was performed. This operation will be described, as performed by us, in another publication concerned with the results of denervation in patients suffering from nephritis. The operation went smoothly and recovery was uneventful. The kidney was found to be normal in appearance. Sections of muscle removed from the lumbar muscles showed, according to Dr. C. P. Rhoads, very little intimal change.

Following operation no change in renal function or blood pressure level was observed. About one month later the left kidney was denervated. Again no significant change occurred in the renal function measured by the urea clearance test or the ability to concentrate. A slight fall occurred in the average diastolic pressure. This was not considered significant in view of the fact that the patient had been subjected to two major operations. Shortly after operation there was a slight increase above normal in the number of red blood cells excreted in the urine, but the hematuria quickly subsided. No change was observed in the eyegrounds as the result of the operations. The hemoglobin also did not change significantly.

An attempt was made by Dr. O. Lowsley to catheterize the ureters after the first operation. The bladder was found to be normal, but the catheters could not be inserted more than five centimeters into the ureters presumably because of spasm. It is interesting to note that on the denervated side the patient felt no pain following cystoscopy. Conversely, on the innervated side the pains were crampy and intense.

The patient felt as well after the two operations as before. No symptoms such as polyuria, nocturia or urgency appeared. However, it cannot be said that she was improved.

#### DISCUSSION

Since the level of arterial blood pressure did not fall significantly following bilateral denervation of the kidneys, it was concluded that in this patient suffering from severe essential hypertension

nervous impulses originating in the kidneys did not share in the maintenance, and probably therefore not in the genesis of the hypertension. The results in this typical case were so decisive that they make it appear doubtful that the kidneys play any rôle, as a general thing, in the genesis of essential hypertension.

Renal denervation was first performed in man by Papin and Ambard (4) for relief of pain. Quinby (5) (1916), Milliken and Carr (6) (1925) and Caldwell, Marx and Rowntree (7) (1931) have presented comprehensive reviews of the literature. Patients suffering from essential hypertension have not heretofore been subjected to renal denervation.

Evidently bilateral denervation is not an operation which jeopardizes renal function. Neither the urea clearance nor the ability to concentrate were impaired. Polyuria and fixation of specific gravity, shown to occur by Quinby (5) and Marshall and Kolls (8) in dogs following denervation, were not observed in our patient.

#### SUMMARY

1. Bilateral renal denervation, in a patient suffering from severe essential hypertension, did not change the level of arterial blood pressure, hence our results give no ground for expecting that denervation in cases of essential hypertension is of therapeutic value.

2. No ill effects, either renal or extrarenal, were observed after the denervation. Renal efficiency, as measured by the urea clearance test and the ability of the kidneys to concentrate, was normal before operation and remained unchanged after denervation.

3. Our results do not support the hypothesis that essential hypertension originates in whole or in part in the nervous mechanism of the kidneys.

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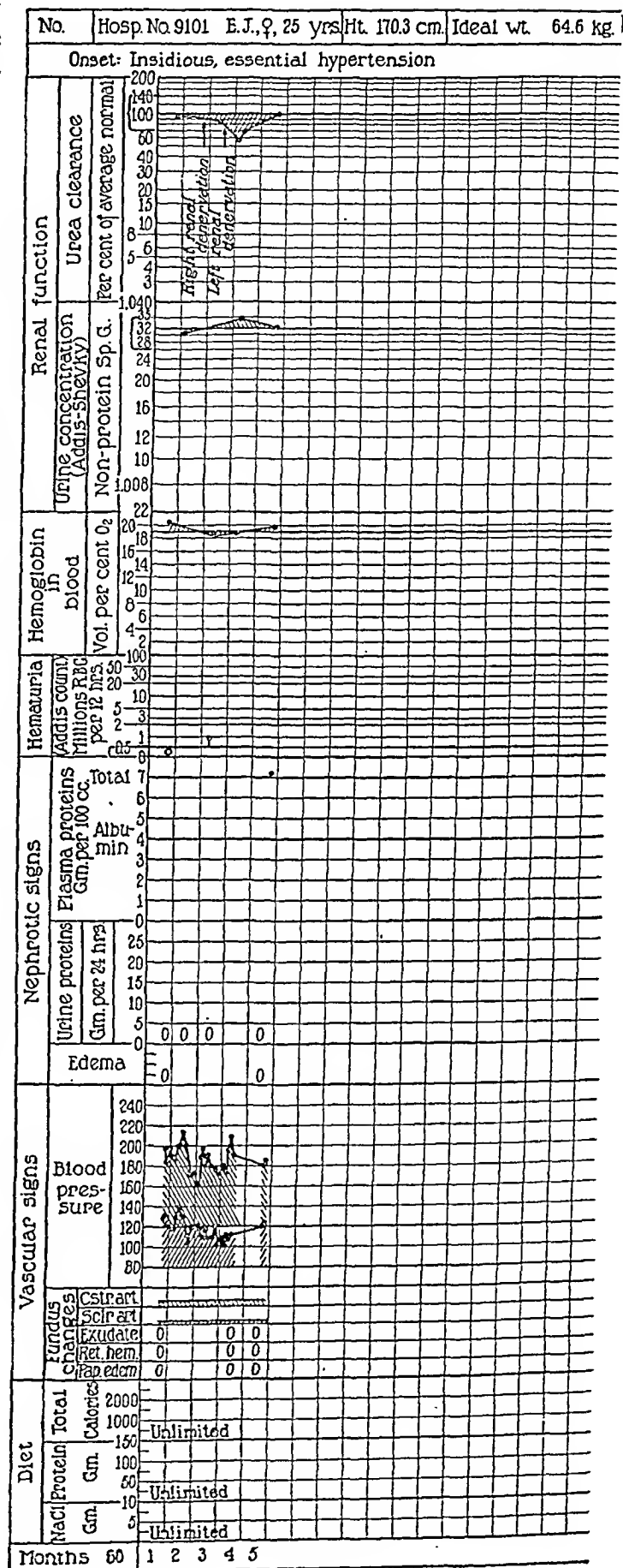


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# SOME EFFECTS OF EXERCISE ON THE URINARY SEDIMENT

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In the course of investigations into various changes produced in the body and its functions by exercise, several workers have studied the urine. Diminution of volume and increase in concentration were early recognized. The occurrence of proteinuria and certain factors related to its occurrence were shown. The fact that urea excretion is impaired by vigorous activity was demonstrated. De la Camp is said to have been the first to note the occurrence of a general increase in the number of formed elements in the sediment. His observations have been repeated several times.

This report gives the first data which are quantitative and which show time relationships.

## METHODS AND MATERIAL

The urine studied was obtained from a group of thirty-one apparently healthy young male athletes. Specimens produced before, during, and after exercise were examined. The exercise was strenuous: football and handball. The duration of the period of exercise varied from forty minutes to four hours. Several series of specimens from three members of the group were studied; one series from each of the others. A series of specimens consisted of (1) a timed specimen pro-

duced in the period before exercise, (2) a timed specimen produced during the period of exercise, and (3) one or more timed specimens produced after exercise. During the periods before and after exercise the subjects were under conditions of ordinary activity, i.e., up and about their usual school duties. No attempt was made to control fluid intake. Specimens from each subject were collected successively and were examined within two hours.

Determinations were made of the rate of excretion of urine, the specific gravity, the rate of excretion of protein, and the rates of excretion of red blood cells, white blood cells and renal epithelial cells, and casts.

Protein was determined by the method of Shevky and Stafford (10). The formed elements in the sediment were counted by the method devised by Addis (1).

## RESULTS

The rate of excretion of urine varies greatly. Most individuals, however, show a reduction in the rate of production of urine while exercising which, upon cessation of vigorous activity, is followed by a rapid return toward the rate existing

TABLE I

*Summary of data for averages shown in figures*

Measurement	Number of subjects	Number of observations	Mean with its standard error		
			Before exercise	During exercise	After exercise
Rate of production of urine, cc. per hour .....	31	41	50.2±10.2	28.5±3.0	48.9±5.2
Specific gravity .....	31	41	1.026±0.00051	1.029±0.00051	1.028±0.00059
Rate of excretion of red blood cells, thousands per hour	31	41	2.98±0.56	3.56±0.65	1.72±0.31
Rate of excretion of white blood cells and renal epithelial cells, thousands per hour .....	31	41	16.73±6.85	71.23±22.98*	23.03±3.83
Rate of excretion of casts, thousands per hour .....	31	41	2.09±0.09	37.9±11.16	10.00±3.83

\* One widely divergent observation (262) has been excluded. If it is included this figure becomes 75.88±42.96

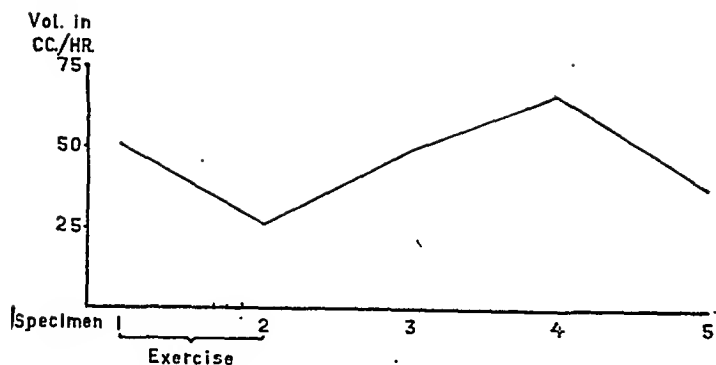


FIG. 1. EFFECT OF EXERCISE ON THE RATE OF PRODUCTION OF URINE.

under conditions of ordinary activity. Figure 1 represents the average response of the group.

The specific gravity increased during the period of exercise, Figure 2. Here again the individual

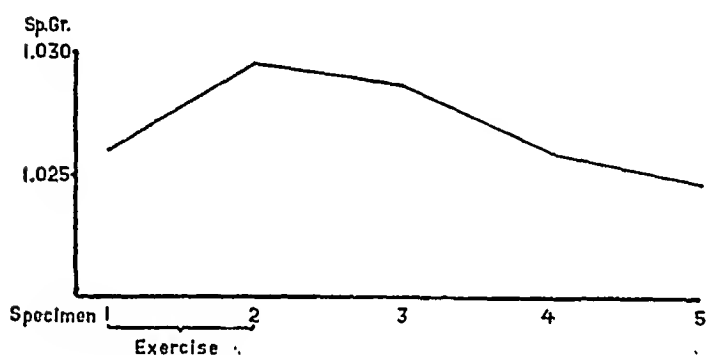


FIG. 2. EFFECT OF EXERCISE ON THE SPECIFIC GRAVITY OF THE URINE.

variation was great. As pointed out by Barach (4), in the individual no correlation seems to exist between the change in rate of excretion and the change in specific gravity; a fall in the volume per hour was often associated with a lower specific gravity.

The effect of exercise on the rate of protein excretion, Table II, was inconstant. It varied in different individuals and in the same individual on different occasions. In one subject who had a functional proteinuria, on some occasions the rate of protein excretion increased; on other occasions it decreased.

The number of red blood cells found in the urinary sediment was unaffected by exercise, Figure 3.

In the number of white blood cells and renal epithelial cells appearing in the urine, exercise produced approximately a four-fold increase, Figure 4. In only one instance, however, did the

TABLE II

*The rate of excretion of protein in the urine*

Number of observations	Rate in mgm. per hour		
	Before exercise	During exercise	After exercise
14	0	0	0
1	0	1.7	0
1	0	3	3
1	0	4	0
1	0	4.5	0
1	0	4.8	6
1	0	12	3
1	0	21.8	0
1	0	30	28.8
1	0.8	0.4	0
1	1	0	0
1	1.4	0	0
1	2	2	2
2	2	0	0
2	2.4	0	0
2	3	2	0
1	3	6	14
1	4	7	1
1	4	3	1
1	4.4	4	3
1	6	0	3
1	8	2	4.5
1	11	0	3
1	14	54	3.8
1	29	140	32

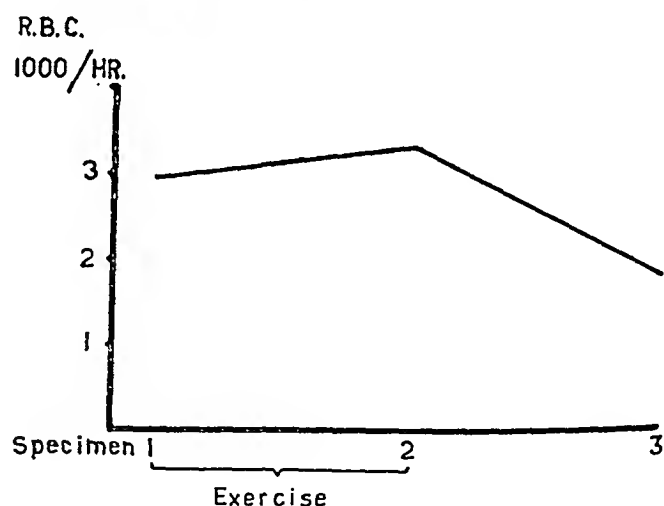


FIG. 3. EFFECT OF EXERCISE ON THE INCIDENCE OF RED BLOOD CELLS IN THE URINE.

number of white blood cells and renal epithelial cells exceed the upper limit of normal (Addis). Return to low levels occurred in every instance, usually within three hours.

Exercise produced a great increase in the number of casts found in the urine, Figure 5. In every observation the number of casts excreted during exercise greatly exceeded the upper limit of normal. The casts were largely hyaline, but occasional granular casts were encountered. No

cellular or blood casts were found. The increase in the number of casts excreted persisted for as long as twelve hours after cessation of activity.

in the rate of excretion of red blood cells. Recovery occurs within a few hours after activity is curtailed.

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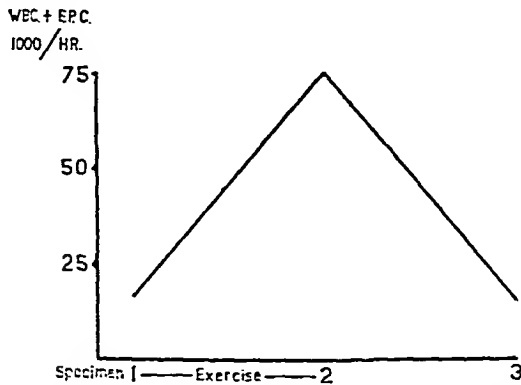


FIG. 4. EFFECT OF EXERCISE ON THE RATE OF EXCRETION OF WHITE BLOOD CELLS AND RENAL EPITHELIUM IN NORMAL URINE.

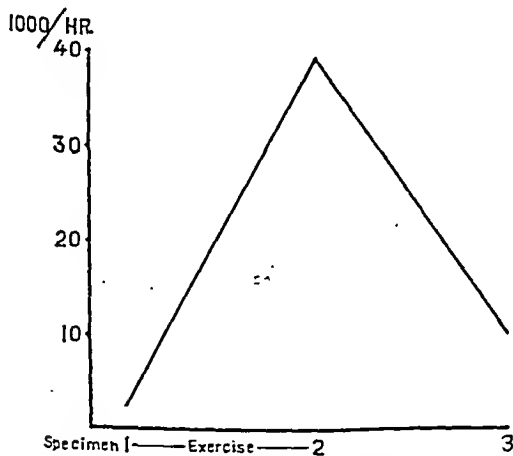


FIG. 5. EFFECT OF EXERCISE ON THE RATE OF CAST EXCRETION.

#### CONCLUSION

Vigorous exercise produces a marked increase in the rate of excretion of hyaline casts, a moderate increase in the rate of excretion of white blood cells and renal epithelial cells but no change



# THE PROTEIN CONTENT OF SUBCUTANEOUS EDEMA FLUID IN HEART DISEASE <sup>1</sup>

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The principal mechanism in the production of edema in heart failure has been considered to be an increase in the capillary pressure (1, 2). Additional factors, possibly of minor importance, are increased capillary permeability due to anoxemia and slight lowering of plasma colloidal osmotic pressure as a result of decrease of plasma proteins. These have been emphasized by Landis and Krogh (3, 4). The general opinion, however, is that lowered colloidal osmotic pressure is not a constant finding in heart disease (5). Landis (3, 4) demonstrated increased protein filtration in the capillaries of the frog mesentery after oxygen deprivation, and by indirect methods showed the same thing in the arm in man. The protein content of the transudate in Landis' experiments averaged 1.5 per cent. Vančura (20) presents analyses in support of the idea that there is considerable increase in permeability of the capillary wall to protein in chronic heart disease. Haas (6) attributes edema formation to disturbances in the "vital function" of the capillary endothelium with consequent alterations in permeability. He states that in circulatory edema the protein content of the fluid varies directly with the extent of the edema. Drinker (7, 8) believes that the normal capillary transudate is similar to lymph in composition, containing 1 to 4 per cent of protein. He also states that the lymph flow in circulatory edema is even greater than normal, the capillary filtrate presumably changing but little in composition.

Because of the high protein values for capillary transudate in normals (Drinker), after congestion and anoxemia (Landis), and because of the variation in many of the reported figures on cardiac edema fluid, most of which were obtained by methods not now considered reliable, a reinvesti-

gation of the protein content of the fluid seemed likely to be of interest.

The reported analyses of the protein content of subcutaneous edema fluids in heart disease are shown in Table I.

TABLE I

*Reported analyses of the protein content of subcutaneous edema fluids in heart disease*

	Protein per cent
Hoppe (9) .....	.36
Reuss (10) (heat and acid pptn.) .....	1.15
	.52
	.56
Senator (11) (heat and acid pptn.—4 cases) .....	.20
	.20
	.16
	.28
	.35
	.34
	.49
	.56
	.60
	.58
	.93
	.50
	.27
	.10
	.13
Hoffman (12) (alcohol pptn.) .....	.06
	.12
	.17
	.17
	.23
	.27
	.27
	.3
	.41
	.43
	.44
	.55
	2.08
Epstein (13) .....	.462
	.119
	.10
Falta (14) (Kjeldahl N × 6.25) .....	.14
	.13
	.19
	.29
Vančura (20) Average (? refractometric)	.610
Beckmann (15) (refractometric) .....	.65
	.60
	.60
	.70
	.37
	.34

<sup>1</sup> Supported by a grant from the Rockefeller Fluid Research Fund.

TABLE I—continued

	Protein per cent
	.49
	.42
	.64
	.25
	.71
	.41
	.37
	1.02
	1.18
	.35
Fodor and Fischer (16) (Kjeldahl) .....	.16
	.34
Salveson and Linder (17) (Kjeldahl) ...	.24
	.35
	.29
Gollwitzer-Meier (18) (refractometric) .	.70
	.1
	.10
Landis (3) .....	.39
	.09
Kylin (19) (refractometric—3 cases) ....	.47
	.47
	.44
	.50
	.42
	.24
	.45
	.55
	.77
Haas (6) (Bang micro method) .....	.187
	.237
	.062
	.025

## METHOD

For the present analyses the edema fluid was collected by means of Southey tubes from the legs of patients who had congestive heart failure but no significant degree of anemia or renal failure. Blood samples were taken simultaneously for protein and other determinations. Protein nitrogen determinations were made in duplicate using Howe's (21) method. Because of the small quantity of protein in the fluids fractional precipitation was not attempted, but a number of the fluids mixed with 22.2 per cent sodium sulfate (Howe) gave no precipitate or at most a very faint turbidity indicating an almost complete absence of globulin by this method of testing.

The results obtained from the analyses of 26 fluids from the same number of patients are given in Table II.

The plasma protein values are lower than normal and are of the same order as those reported in heart disease by Payne and Peters (22). While the protein values for the edema fluid show considerable variation, they tend to be lower than most of those previously reported except for a

TABLE II

## Plasma protein and edema fluid protein from 26 patients

Patient	Plasma protein grams per 100 cc.*	Edema fluid protein grams per 100 cc.*
1 .....	5.76	.16
2 .....	4.95	.14
3 .....	6.29	.08
4 .....	5.14	.12
5 .....	5.73	.54
6 .....	5.92	.05
7 .....	5.5	.07
8 .....	5.5	.13
9 .....	6.2	.24
10 .....	5.94	.20
11 .....	5.64	.14
12 .....		.33
13 .....	4.73	.03
14 .....	5.7	.19
15 .....	5.01	.11
16 .....	4.85	.21
17 .....	5.7	.10
18 .....	6.06	.36
19 .....	6.02	.05
20 .....	5.09	.30
21 .....	5.49	.43
*22 .....	5.02	.35
23 .....	6.41	.35
24 .....		.36
25 .....		.14
26 .....		.22

\* N  $\times$  625.

few Kjeldahl analyses. No relation was noted between either the duration or the extent of the edema and the protein content of the fluid. The low protein content of the fluid would indicate that the degree of capillary damage in the patients studied is insufficient to permit the passage of more than negligible amounts of plasma proteins, and therefore is of little importance as a factor in the production of edema in heart disease. In edematous patients, at least, the fluid in contact with the capillary wall is probably of the same composition as that removed through the Southey tubes, and this "tissue fluid" thus differs markedly from the lymph analyzed by Drinker, and also from the presumptive composition of the capillary transudate estimated indirectly by Landis from his experiments on the effect of venous congestion. This agrees with the idea of Krogh, Landis, and Turner (4) who believe that the fluid bathing the capillaries is of low protein content although it may become more concentrated in protein as it approaches the lymph channels.

It is also to be expected that such small quantities of protein would exert no significant colloidal osmotic pressure to counteract that of the blood proteins, and that the effective colloidal osmotic pressure between the intracapillary and extracapil-

lary fluids in edematous patients would approach but not quite equal that between plasma and its protein-free filtrate as measured in vitro.

#### SUMMARY

1. Concentrations of protein in subcutaneous edema fluids from 26 patients with congestive heart failure varied from 0.03 to 0.54 per cent with a mean value of 0.21 per cent.

2. The low protein concentration in cardiac edema fluids indicates that the factor of capillary damage from anoxemia or other cause is not of importance in the production of cardiac edema.

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# THE CALORIGENIC ACTION OF D- AND L-THYROXIN

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(Received for publication August 14, 1934)

Evaluation of the significance of calorigenic responses obtained by the assay of constituents of the thyroid in myxedematous persons is fraught with difficulty. The questions of absorption, excretion and destruction of the material administered all intrude to plague the investigator. The rôle of physical properties such as solubility or optical activity must be identified. The possibility of enhancement of physiologic potency brought about by alteration in physical state has been mentioned in previous communications (1) (2). In the first paper of this series (1) it was shown that racemic crystalline thyroxin and optically active thyroxin polypeptide had equal calorigenic activity when given in equal iodine dosage.

To settle, if possible, the single point of the part played by optical activity the present study was undertaken. We are indebted to Professor C. R. Harington for a supply of pure crystalline *d*- and *l*-thyroxin (3). The substances have been assayed on myxedema patients in the manner described in earlier papers (1) (2). The results are shown graphically in Figure 1. A curve which represents the average response obtained in five myxedema patients receiving 1.0 mgm. of thyroxin polypeptide (50 per cent iodine) per day by mouth is also included in this figure in order to indicate the standard response to 0.5 mgm. of thyroid iodine daily.

Special care was taken to avoid racemization of the optically active material. The weighed micro-crystalline powder was dissolved in cold 0.01 normal sodium hydroxide, of which 6 cc. were used per milligram of iodine. Three cubic centimeters of the freshly prepared thyroxin solution were measured into sterile ampoules. To each ampoule was added 1 cc. of distilled water and enough fifth-normal orthophosphoric acid to bring the reaction of the solution to pH 6.5. The ampoules were then stoppered with special "bacteriological" rubber caps, shaken to disperse the precipitated thyroxin, and heated at 80° C. for ten

minutes. They were then kept iced until used. Three or four such ampoules were prepared at once, to be used on successive days. Immediately before injection, one-half cubic centimeter of cold tenth-normal sodium hydroxide was added by means of a sterile syringe and the thyroxin again dissolved by shaking.

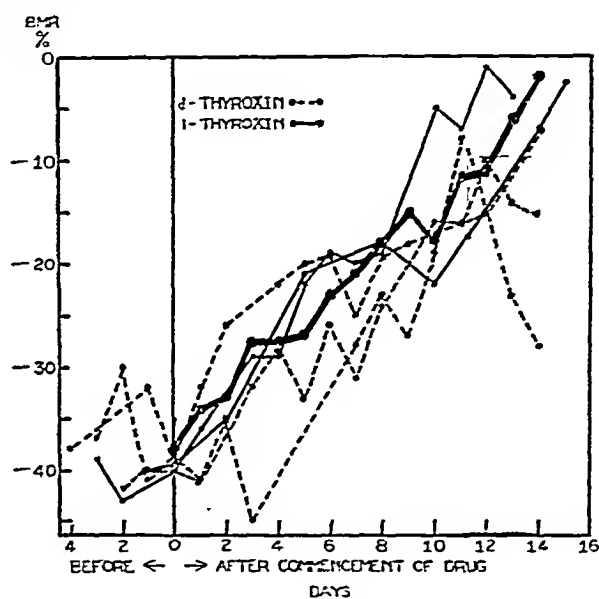


FIG. 1.

The calorimetric responses in two patients with spontaneous myxedema receiving a daily intravenous dose of 0.75 mgm. of *l*-thyroxin (light solid lines), and of three patients receiving a daily intravenous dose of 0.75 mgm. of *d*-thyroxin (interrupted lines). The solid curve represents the composite of five assays of thyroxin polypeptide given in daily oral doses containing an equivalent amount of iodine, namely 0.5 mgm.

In this way, the racemizing effects of heat, alkali, and strong acid were kept at a minimum. These precautions are as rigorous as those used by Harington and Salter (4) in the isolation of *l*-thyroxin.

Two assays were obtained with *l*-thyroxin and three with *d*-thyroxin. The subjects were all untreated cases of spontaneous myxedema, none of

whom were used for more than one assay. Each preparation was given intravenously once daily in doses of 0.75 mgm. daily this being equivalent in iodine content to the 1.0 mgm. of the control polypeptide assays.

The two *l*-thyroxin assays and two of the *d*-thyroxin assays are sufficiently close to the control curve to be considered to represent identical responses. One of these two patients receiving *d*-thyroxin also received desiccated thyroid, 3.75 grains daily by mouth (0.5 mgm. organic iodine) at the end of the series of thyroxin injections. The metabolism rose from minus 15 per cent to 0 per cent in six days, but the slope of the curve remained the same. The third patient receiving *d*-thyroxin began to make a standard response, the metabolism reaching minus 8 per cent on the eleventh day, but subsequently dropped to minus 28 per cent on the fourteenth day. When desiccated thyroid 3.75 grains daily (0.5 mgm. organic iodine), was substituted two days after the last thyroxin injection, the metabolism changed relatively little, reaching minus 18 per cent after seven days of medication. Consequently the low response yielded by this patient receiving the dextrorotatory isomer may be discounted as due to some peculiarity inherent in the patient and not in the substance assayed. These measurements seem to indicate that the response of myxedema patients to either optical isomer of thyroxin is essentially identical.

#### COMMENT

This series (1) (2) (4) (6) of observations was begun in an attempt to explain the high metabolic potency of thyroglobulin in which thyroxin itself may constitute only a minor portion of the total iodine. It was originally suspected that peptide linkage and optical activity (4) (7) might be the chief factors involved in the high potency of the natural hormone. As previously reported (1), thyroxin in natural peptide combination failed to show unexpectedly high activity. This result has been confirmed by observations to be reported elsewhere upon racemic glycyl thyroxin (8) kindly donated by Harington. These synthetic products embodying peptide linkage and optical activity have enabled us to analyze the effects of the two characteristics separately. Neither of these chemical relationships has been

found to alter significantly the activity of thyroxin provided that absorption was ensured by parenteral administration.

It has become evident, however, that the observed potency of the drug depends to a considerable extent upon the mode of assay. For example, it has been known for a long time (9) that the intact thyroid tends to trap iodine if the latter is in excess and to withhold it from the systemic circulation. Similarly Schittenhelm and Eisler have shown (10) (11) that when a single large dose of thyroxin is administered, much of the iodine is excreted in the first forty-eight hours. Such a condition is obviously abnormal, and one is constantly faced with the question: does our assay represent a physiological potency, or is it merely the expression of the net result of an experiment under complex pharmacological conditions? Assays of thyroid activity have been made both in man (1) (2) and in animals (13) by the method of massive dosage. Such procedures, we believe, induce disturbances of body mechanisms, which are foreign both to the physiological behavior of mammals and to ordinary routine clinical conditions. Such abnormally planned procedures, it would seem, have tended to mislead us not only as to the action of the thyroid hormone under normal conditions but also as to its effect in the usual cases of myxedema as treated in present-day clinics.

Criticism on the grounds mentioned above is avoided by the method of assay here employed, which consists of the administration of a uniform daily dose of thyroid material at a rate calculated to restore the patient to a nearly normal state in some two weeks' time. This is a therapeutic program in use in many clinics at the present date. Certain it is, that this procedure resembles the usual clinical methods of thyroid therapy more closely than does the administration of massive single doses of the drug.

The results here reported are at variance with assays in the literature which show that the levorotatory isomer is several times as potent as the dextrorotatory. Of these researches we select for comment only one, that of Gaddum (13), because the assays were made with the identical preparation used by us and supplied by Harington. His observations, made upon normal rats to which single large doses of thyroxin (*d*- or *l*-, re-

spectively) were administered, showed the *l*- compound to be about three times as active as the *d*- isomer. The difference between his results and ours, we believe, may be explained upon several grounds. In the first place, the animals possessed an intact thyroid apparatus which might trap newly-administered iodine, and which might fluctuate in activity. Secondly, the drug was administered in a single massive dose (4 to 10 mgm. of thyroxin per kilo) as against our daily dose in man of 0.01 mgm. per kilo) so that rates of excretion and destruction of the drug would play important rôles in determining the duration and height of the responses. In fact, according to Gaddum's own data, the discrepancy between the two isomers became smaller with smaller doses than with larger doses. Whether the difference in species contributed to the discrepancy we do not know. We merely conclude that such observations, however valid as laboratory experiments, can not be expected to apply quantitatively to the reaction of the human case of myxedema.

#### CONCLUSIONS

When assayed by a method designed to reproduce approximately both physiological and ordinary clinical conditions, *d*- and *l*-thyroxin were found to have essentially identical potencies in relieving human myxedema.

This fact lends additional support to the thesis that the high potency of thyroglobulin is not due to a super-active form of thyroxin.

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# EXPERIMENTAL STUDY OF CLINICAL VITAMIN B DEFICIENCY<sup>1</sup>

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Clinical disturbances incident to mild deficiency of vitamin B<sup>2</sup> in the diet, as opposed to frank deficiencies such as beriberi and pellagra, are commonly believed to be very rare in man and are thought to occur only when the diet is grossly abnormal or when there is serious interference with absorption from the gastro-intestinal tract. Certain observations made in this Gastro-Intestinal Section, however, have suggested that clinical evidences of such partial deficiency may develop on

given for the first five months a diet moderately deficient in vitamin B though adequate in every other known requirement and the effects of this diet were studied. When clinical signs suggestive of deficiency were manifest, various vitamin B fractions were added to the diet, in series, and the effects of their administration studied. The second patient (Case 2), who entered the hospital with fully developed clinical evidence of deficiency, was likewise placed on a diet restricted in

TABLE I  
Chart showing articles composing the diet deficient in the vitamin B complex

Cereals	Meat	Vegetables
Cream of wheat	Lamb	Potatoes: white sweet
Rice	Veal	Turnips
White cornmeal	Beef (50 grams twice weekly)	Corn
		Spinach
		Onion
		Beets
		Cabbage
		Rutabaga
		Carrots
		} Boiled 2 hours
Fruit	Pastry and desserts	Beverages
Orange juice: 100 grams daily	Raisin pudding	Tea
	Cornstarch pudding } without milk	Coffee
	Jello	
Apple	Pie: apple	
Pear	pear	
Grape	Cake } with white flour	
Cocoanut	Biscuits } and without milk or egg	
	Jelly: grape	
	apple	
Fats		Seasonings as desired
Butter		Chocolate, cinnamon,
Cod liver oil: 10 grams daily		molasses, salt, etc.
Lard		

a diet which is not obviously abnormal and in the absence of serious gastro-intestinal disease. Experiments to test this hypothesis were carried out on two patients, one of whom (Case 1) was hospitalized for approximately a year. She was

its content of vitamin B and was studied during the addition to it of separate vitamin B fractions.

## METHODS OF STUDY

The articles of which the experimental diet was composed are recorded in Table I.<sup>3</sup>

<sup>1</sup> Aided by a grant from the Faculty Research Committee of the University of Pennsylvania.

<sup>2</sup> The term vitamin B as used in this paper includes all members of the vitamin B complex; individual members are referred to as B<sub>1</sub>, B<sub>2</sub> (G), and the "additional factors": B<sub>4</sub>, B<sub>5</sub>, and factor Y.

<sup>3</sup> The vitamin B<sub>1</sub> content of the diet for Case 1 was calculated by Dr. George Cowgill according to a formula of his as yet unpublished, and was found to contain approximately one-half of the theoretical vitamin B<sub>1</sub> requirement.

All food was weighed. Caloric, fluid and protein intake was constant throughout the entire experiment, the latter intake amounting to 49 grams per day for Case 1, and 68 grams for Case 2. Daily output of urine was measured. Symptoms and physical signs were recorded daily. Mouth temperature was taken morning and evening. The patients were weighed daily under standard conditions on a balance accurate to within 8 grams. Nitrogen balance was determined during 2 three-day periods, both food and excreta being analyzed. Roentgen examination of the gastrointestinal tract was made at the beginning and end of each experimental period; observations of the level and tone of the stomach, of the type of peristalsis, and of the motility throughout the small intestine and colon were made every two hours for six hours. Fractional gastric analyses were carried out using oatmeal gruel as the test meal. Exercise tolerance tests (1) were performed during each experimental period. Blood pressure and pulse rate were measured under basal conditions and repeated on standing and after climbing 54 steps at maximal speed. Serum protein analyses, enumeration of red blood cells, hemoglobin estimations (2) and hematocrit readings, carried out under conditions which insured complete packing of cells, were made weekly. Venous pressure was measured from accessible veins on the dorsum of the hand by the indirect method of Krogh, Turner and Landis (3). Determination of capillary permeability was made in Case 1 at the height of the edema using the procedures described by Landis, Jonas, Angevine and Erb (4). Respiratory quotient, basal metabolic rate, electrocardiograms, orthodiagrams, tests of renal function, platelet counts, sugar tolerance tests and estimations of coagulation time were made in the routine hospital laboratories.

The vitamin B<sub>1</sub> substance<sup>4</sup> was prepared as an 80 per cent alcoholic extract of wheat embryo according to the method of Bourquin and Sherman (5). Using the rat growth method of biological assay (6), this extract was found to be free of other vitamin fractions and to be potent in vitamin B<sub>1</sub>, 1 cc. of the extract containing approxi-

mately 21 Sherman units. The required dose of this substance for Case 1, 11.3 cc., was calculated from the optimal dose in rats on the basis of relative caloric requirements. To be certain of adequate dosage 20 cc. were given daily for 3 weeks, after which the daily dose was increased to 40 cc. The vitamin B<sub>2</sub> concentrate was made from egg white by the method of Chick, Copping and Roscoe (7). It was found to be potent in vitamin B<sub>2</sub> and free of other vitamin fractions. The calculated human dose was first doubled, 60 cc. of the extract being given daily for 2 weeks, and then increased to 120 cc. for the remainder of the experiment. Powdered brewer's yeast concentrate<sup>5</sup> prepared by a modification of the Osborne and Wakeman method (8) and known to contain factors in addition to vitamins B<sub>1</sub> and B<sub>2</sub> necessary for maximal growth in the rat (Figure 2) was used as a source of additional B factors. An arbitrary dose of 6 tablespoonfuls was given daily. An extract of rice polishings suitable for parenteral use,<sup>6</sup> shown (9) to be a potent source of vitamin B<sub>1</sub> was used for a short time in Case 2; the daily dose employed varied from 2 to 4 cc.

#### PROTOCOL

*Case 1.* A woman, 56 years old, 158 cm. tall, weighing 57.2 kilos, had been admitted one year prior to hospitalization as an out-patient to this Section, complaining of anorexia, sore tongue, asthenia, paresthesias in the lower extremities, dyspnea and vertigo. She at that time gave a history of voluntary restriction of diet for 10 years and, as described elsewhere (10), had a bald tongue, exaggerated patellar reflexes, impaired vibratory sensation and edema of the lower extremities. Blood studies and gastric analysis were negative. Whole yeast concentrate was administered at that time for 6 weeks, following which the patient had become symptom free and was then without physical signs except exaggerated reflexes and diminished vibratory sensation in the lower extremities. She had continued in good health throughout the remainder of that year. It was then decided to determine the effect of moderate limitation of diet at home. This was done for 6 weeks prior to hospitalization with the result that on admission some impairment of appetite, slight intermittent edema of the ankles, and occasional sore tongue were manifest. The strict experimental diet was begun immediately upon admission,

<sup>4</sup> Obtained from the Harris Laboratories, Tuckahoe, New York.

<sup>6</sup> This material was kindly supplied through the courtesy of Dr. A. W. Rhodehamel of the Eli Lilly Research Laboratories.

<sup>5</sup> Vitamin materials were prepared and assayed in the Laboratory of Physiological Chemistry under the direction of Dr. J. H. Jones, to whom acknowledgement is gratefully made.



May 17, 1933, and was continued until March 29, 1934. For a period of 21 days in August 1933 she was away from the hospital but continued the diet. The times at which the various vitamin fractions were added to the diet together with various other data from the case may be seen in Figure 1.

ently been taking an adequate diet in addition to considerable quantities of beer, but had induced vomiting several times weekly to relieve abdominal distress. Constipation persisted, and dyspnea, vertigo, palpitation and edema had developed. Cardiac and renal studies were negative. Free hydrochloric acid was obtained from the

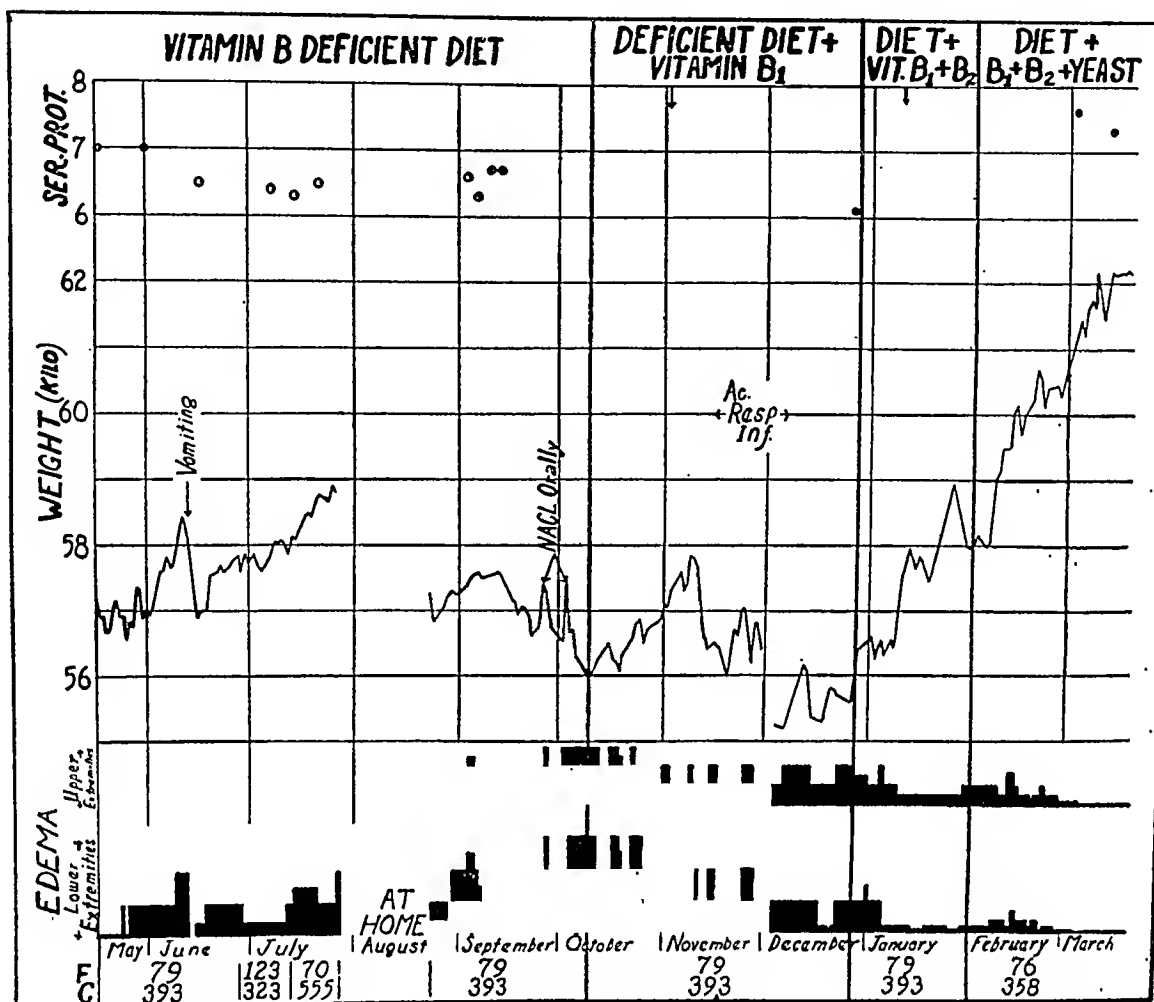


FIG. 1. CASE 1. CHANGES IN WEIGHT, EDEMA AND SERUM PROTEIN ON VITAMIN B DEFICIENT DIET AND FOLLOWING ADMINISTRATION OF VITAMIN B FRACTIONS.

Arrows at the top of the chart indicate doubled dosage of vitamin B<sub>1</sub> and B<sub>2</sub> preparations respectively. Daily intake of fat and carbohydrate in grams is recorded at the bottom of the chart.

**Case 2.** A man aged 53 years, weighing 65.1 kilos at the beginning of observation, had had a long history prior to this admission. In 1924, when first seen in the hospital, he complained of epigastric discomfort after eating, and of nausea, vomiting and constipation. Roentgenological study at that time revealed extensive gastric polypoid and repeated examinations subsequently showed no change. Gastric analysis, routine blood examinations and all other laboratory studies were negative in 1924. In 1927 the symptoms had not changed, except that epigastric distress had become sufficiently severe to cause the patient to induce vomiting. All studies were negative except those of the blood, which showed red cells numbering 3,450,000 and hemoglobin of 85 per cent. In 1930 the patient again entered the hospital. He had appar-

stomach only after histamine. Peripheral reflexes were exaggerated. Red blood cells numbered 3,000,000; the hemoglobin was 64 per cent. On November 13, 1933, he was again admitted to the hospital because of alternate attacks of constipation and diarrhea, the latter persisting several weeks at a time, with 10 or more movements daily. His appetite was good, although his dietary habits, the induced vomiting, and the epigastric discomfort were unchanged. Dyspnea, palpitation and vertigo had increased. He complained of being easily fatigued. The tongue was smooth and red. Edema extended to the knees. Tests of renal function and orthodiagram were negative. An electrocardiogram showed moderate tachycardia. Blood pressure was 102/68. He complained of "crawling sensations" in the feet and legs. Reflexes

were exaggerated and vibratory sensation greatly impaired in the lower extremities. Other neurological findings were normal. Gastric acid was present in moderate amounts without histamine. Red blood cell count was 3,700,000 with hemoglobin of 75 per cent. Serum protein was 4.97 grams per 100 cc. blood. Blood sugar determinations and glucose tolerance tests gave normal figures. He remained on the regular ward routine for 19 days, during which time no change occurred in symptomatology, physical signs or laboratory findings. Following this period the experimental observations were begun (see Figure 6). Diarrhea, induced by sulphates used in preparation of the egg white extract, made it necessary to discontinue the administration of that substance. Yeast therapy was begun immediately and the patient was therefore not observed on vitamin B<sub>1</sub> and B<sub>2</sub> therapy alone. The B<sub>2</sub> content of the yeast was supplemented by 5 eggs daily. Believing that there might be interference with absorption from the gastro-intestinal tract the vitamin B<sub>1</sub> preparation for parenteral use described above was given intravenously. After 7 weeks on the experimental régime he left the hospital, continuing the diet and vitamin therapy. He returned 3 times weekly for observation and intravenous administration of the vitamin B<sub>1</sub> preparation.

## RESULTS

*Case 1.* The symptoms and physical signs which developed in the course of the experiment are outlined in Table II. All of the findings listed in the period "deficient diet" had developed within the first 5 weeks of that period, after which

there was a gradual increase in their severity. During the period of deficient diet plus administration of vitamin B<sub>1</sub> improvement was first observed 2 weeks after therapy was begun. Midway in this period the patient developed an acute upper respiratory infection accompanied by fever. All manifestations of deficiency became aggravated in spite of increased dosage of the vitamin preparation. After subsidence of the infection the improvement noted during the first 2 weeks returned. Attention is directed to the fact that improvement during administration of vitamin B<sub>1</sub> and of vitamin B<sub>1</sub> and B<sub>2</sub> was not continued in certain respects during the end of the latter period and was resumed only when yeast was added to the diet. It is to be noted, also, that symptoms of anorexia and epigastric distress were relieved only when therapy with yeast was begun.

Data relating to body weight, edema and serum protein are presented in Figure 1. It is worthy of note that during the period of vitamin deficiency the body weight decreased while the edema increased, whereas during the periods of vitamin administration increase of weight was accompanied by a disappearance of edema. Serum protein remained above the critical level for edema throughout the entire experiment, but an increase was observed during full vitamin therapy. Nitro-

TABLE II

*Symptoms and physical signs present in Case 1 at the conclusion of each experimental period*

	Pre-hospital period (6 weeks)	Deficient diet (21 weeks)	Deficient diet + B <sub>1</sub> (12 weeks)	Deficient diet + B <sub>1</sub> and B <sub>2</sub> (4 weeks)	Deficient diet + B <sub>1</sub> + B <sub>2</sub> + yeast (8 weeks)
Asthenia.....		Moderate	Improved	No change, later increased	Absent
Sore tongue.....	Slight Moderate	Severe	Severe	Absent	Absent
Anorexia.....		Severe	Very severe	Very severe	Slight
Epigastric distress....		Severe	Very severe	Very severe	Absent
Constipation.....		Severe	Very severe	Slight improvement	Absent
Pains in extremities...		Frequent and severe	Less frequent, less severe	No change, later increased	Infrequent
Paresthesias.....		Frequent	Less frequent	No change, later increased	Infrequent
Average temperature, °F.....	98	97.2	98	98.1	98.2
Tongue.....		Smooth	Partial regeneration papillae	Normal	Normal
Pallor.....		Pronounced	Absent	Absent	Absent
Ecchymoses.....		Numerous*	Absent	Absent, later scattered	Absent
Edema.....	Very slight Exaggerated	Extensive	Decreased	Decreased	Absent
Reflexes.....		Exaggerated	Exaggerated	Less exaggerated	Less exaggerated
Vibratory sensation...		Lost except over internal malleoli	Lost	Perceived over external malleoli	Normal

\* Ecchymoses varied from 1 to 2 cm. in diameter; they were distributed over forearms and legs.

gen balance was found positive when vitamin B<sub>1</sub> was added to the deficient diet. The striking gain in weight observed when the deficient diet and vitamin B<sub>1</sub> and B<sub>2</sub> preparations were supplemented by yeast was duplicated in rats rendered vitamin B deficient on a Sherman basal diet (5) and to which the same preparations were fed in analogous dosage (see Figure 2).

Red blood cell enumeration, hemoglobin and hematocrit readings made throughout the entire experiment are shown in Figure 3. Platelet counts were normal, as was bleeding time. Clotting time, determined on 2 occasions during deficiency, averaged 13 minutes; at the end of vitamin therapy it was 7 minutes.

Blood pressure changes following standard ex-

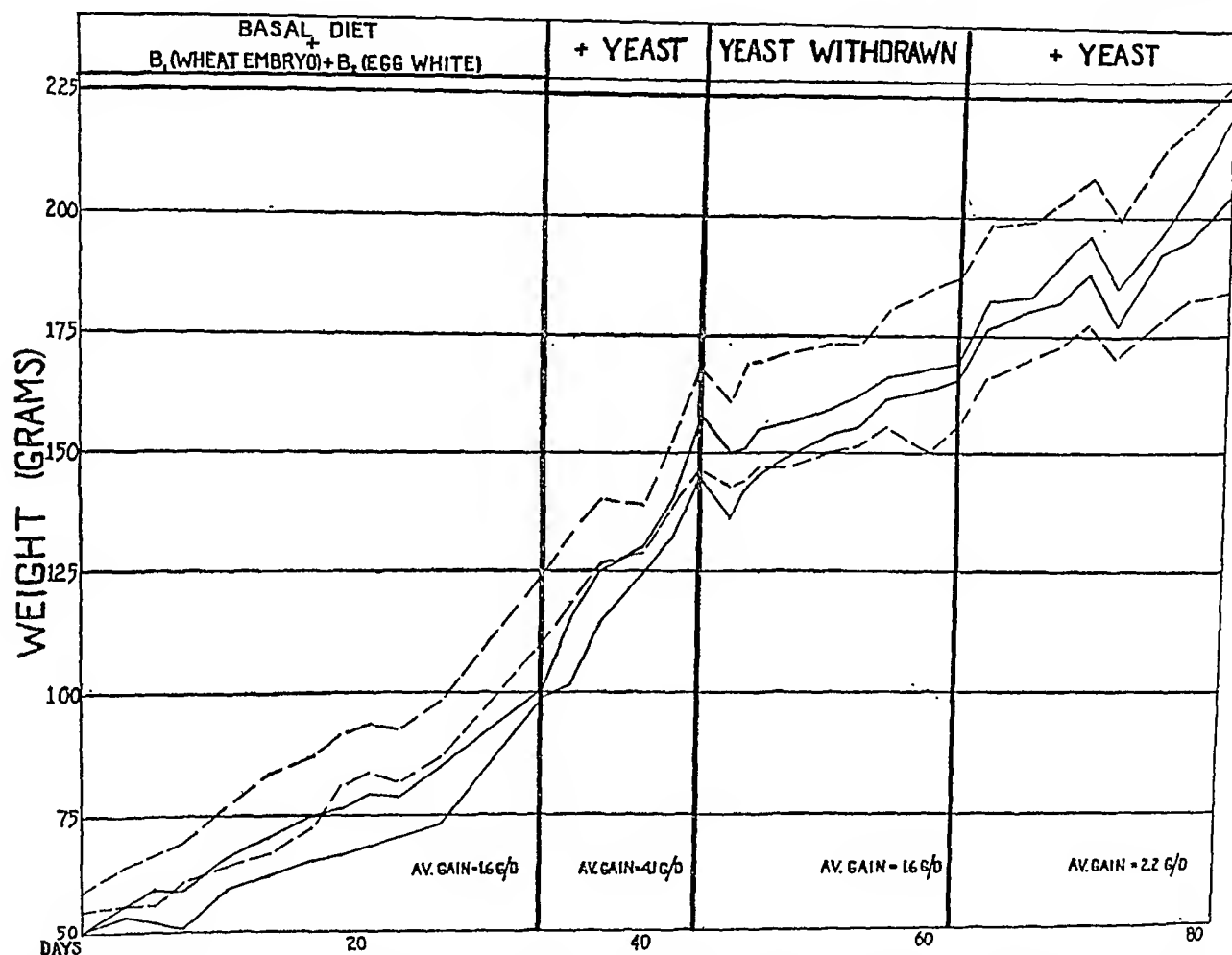


FIG. 2. WEIGHT CURVES OF RATS RECEIVING A SHERMAN BASAL DIET FREE OF VITAMIN B SUPPLEMENTED BY WHEAT EMBRYO, EGG WHITE AND YEAST.

Broken lines indicate growth of animals receiving doubled dosage of egg white.

Venous pressure in Case 1 was between 7 and 10 cm. of water. Measurements of the volume of fluid lost through the capillary wall were made at venous pressure of 67 mm. mercury and the protein content of this fluid calculated. The average calculated volume of fluid filtered in 6 experiments was 10.5 cc. per 100 cc. blood. The average amount of protein in this filtrate was 1.5 gram per 100 cc. These results fall within the normal limits observed by Landis et al. (4), hence they provide no evidence of increased capillary permeability.

Exercise are shown in Figure 4. Preliminary control readings showed a variation of 5 mm. mercury under the standard conditions of the test. The difference in blood pressure before and after vitamin therapy is most clearly seen in the reading obtained after exercise. Systolic pressure at the end of the deficient period fell 10 minutes after exercise to 88 mm. mercury but after vitamin therapy it fell only to 130 mm. mercury.

Electrocardiogram, orthodiagram and tests of renal function were negative.

The results of roentgen examination of the

gastro-intestinal tract are recorded diagrammatically in Figure 5. Gastric atony is represented by failure of the stomach to support the column of barium well into the fundus. This was noted

marked increase of motility in the small intestine; at the end of two hours there was only a small gastric residue, the head of the meal had reached the cecum, the majority of it being in the ileum;

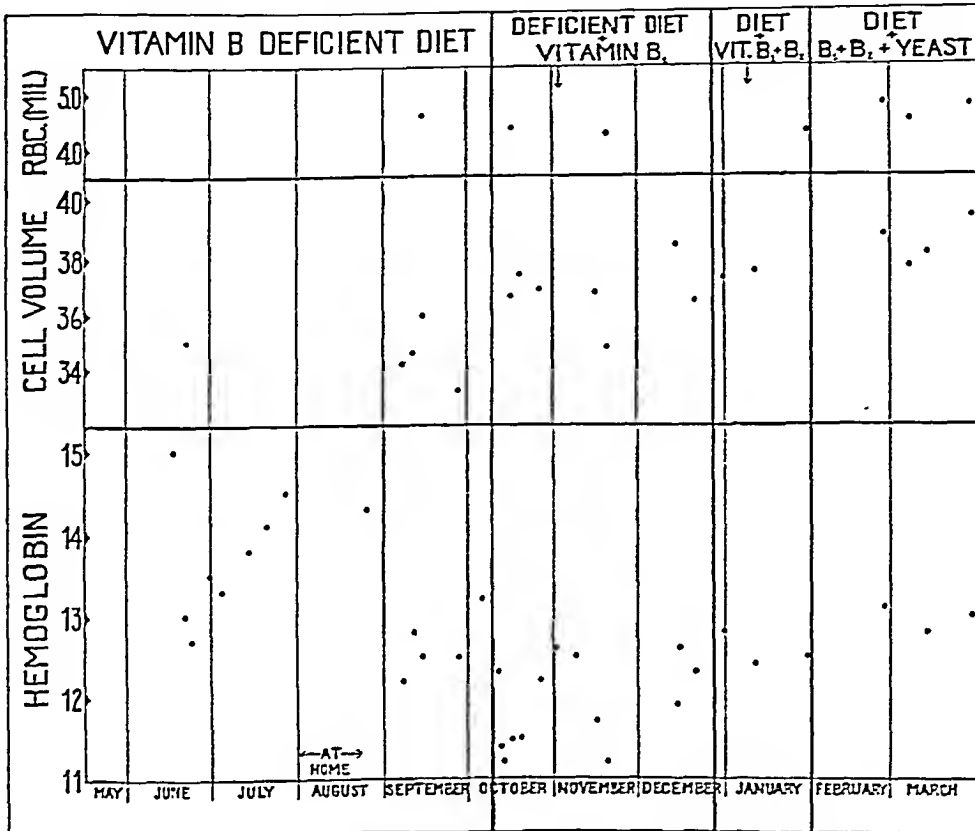


FIG. 3. CASE 1. CHANGES IN HEMOGLOBIN, CELL VOLUME AND NUMBER OF RED BLOOD CELLS ON VITAMIN B DEFICIENT DIET AND FOLLOWING ADMINISTRATION OF VITAMIN B FRACTIONS.

Arrows at the top of the chart indicate doubled dosage of vitamin B<sub>1</sub> and B<sub>2</sub> preparations respectively.

in increasing extent until after the conclusion of the administration of vitamin B<sub>1</sub> and B<sub>2</sub>. Throughout these periods peristalsis was infrequent, and the waves were shallow; at the end of two hours over one-half the meal still remained in the stomach; stasis in the small intestine was evident six hours after giving the barium meal. After yeast therapy there was distinct improvement in gastric tone; the stomach was more globular in shape and supported the column of barium well into the fundus; peristalsis was active and produced prompt emptying of the stomach; ten minutes after giving the barium meal considerable material had passed into the duodenum and had progressed well into the jejunum; there was a

colonic motility was increased, the meal having advanced to the splenic flexure at the end of four hours. These changes in the gastro-intestinal tract seen by fluoroscope took place at the time that the patient noticed relief from epigastric distress, a return of appetite and relief from constipation.

Gastric analysis at the end of the deficient period gave normal values for acid.

Blood sugar on two occasions during deficiency averaged 83 mgm. per 100 cc. blood. Glucose tolerance test gave normal figures. Respiratory quotient, determined with the patient at rest, was 0.77 and 0.78. After 11 days on a high carbohydrate diet the quotient was 0.88. At the end

of combined vitamin therapy it was 0.72. Basal metabolic rate was normal throughout the entire experiment.

4 kilos on addition of yeast to the diet, on the other hand, has been maintained. Anemia did not improve during the control period (see Fig

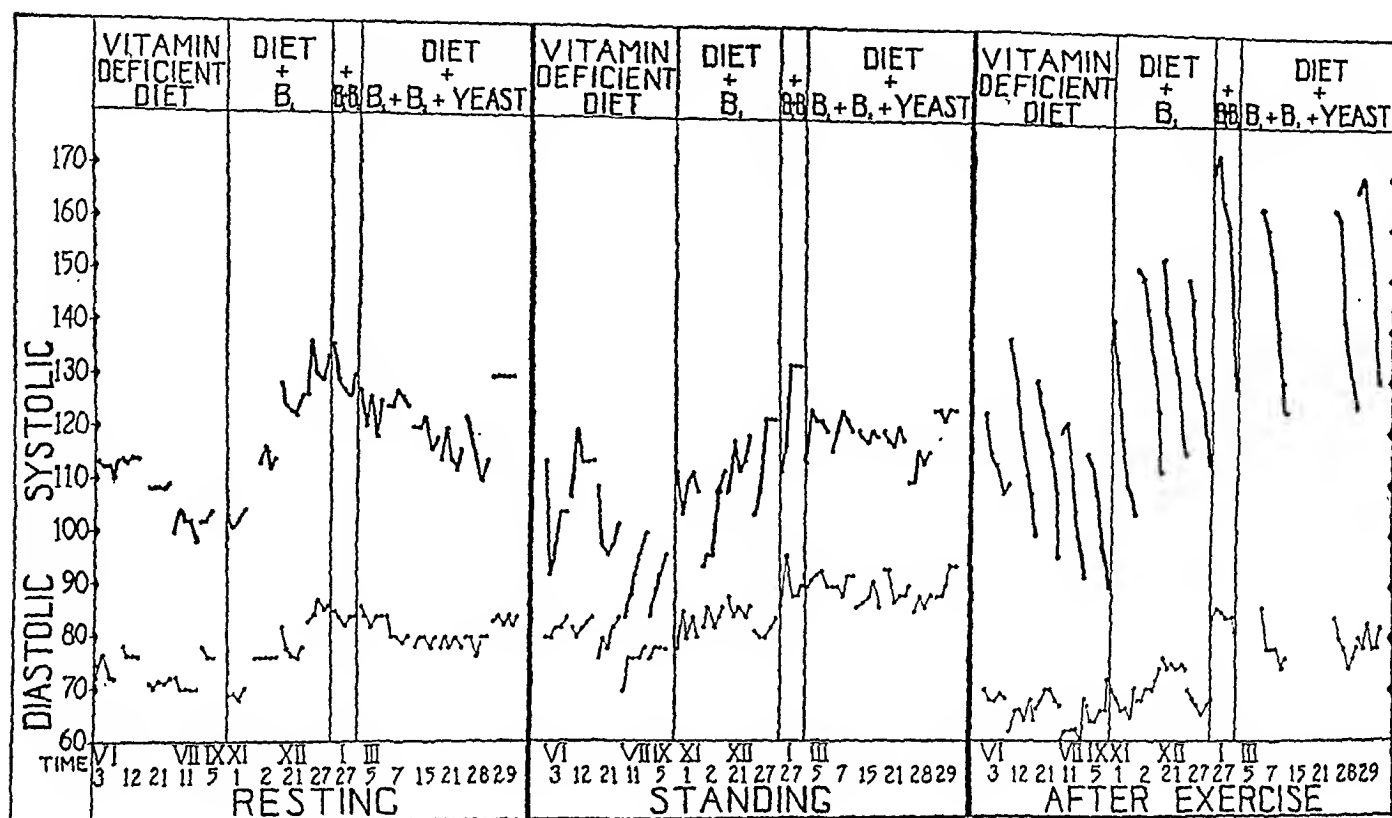


FIG. 4. CASE 1. VARIATIONS IN DIASTOLIC AND SYSTOLIC BLOOD PRESSURE, WHILE AT REST, AFTER ASSUMING A STANDING POSITION, AND AFTER RAPID ASCENT UP 54 STEPS, WHILE ON THE VITAMIN B DEFICIENT DIET, AND DURING THE ADMINISTRATION OF VITAMIN B FRACTIONS.

Each point in a continuous line represents successive readings at 1 minute intervals for 5 minutes. An additional reading was made at the end of 10 minutes after exercise.

**Case 2.** In Figure 6A are shown the results of observations of serum protein, weight, and edema. Total serum protein at the end of the control period was 4.46 grams per 100 cc. blood. Colloid osmotic pressure of the serum calculated according to the formula of Govaerts (11) (12) was 16.2 mm. mercury. When edema first decreased the total protein was 5.17 grams per 100 cc. blood, and when edema was negligible 5.50 grams per 100 cc. blood. The calculated osmotic pressure of the serum at this time was 20.2 mm. mercury. Three and one-half months later, a reading subsequent to the last one recorded on the chart, the serum protein was 6.6 grams per 100 cc. blood and the osmotic pressure 27.2 mm. mercury. Increase of weight on the control diet was coincident with increasing edema. Decline in weight following administration of vitamin B<sub>1</sub> occurred with rapid loss of edema. The gain of

ure 6B). Volume index at this time was 1.14, color index 1.10. During therapy with vitamin B<sub>1</sub> there was an increase in hemoglobin, erythrocytes and cell volume, but no significant change in volume or color indexes. At the end of 3 months of vitamin B<sub>1</sub> and yeast therapy the erythrocytes had increased in number by 34 per cent, the volume index was 0.89 and the color index 0.90. Pulse rate, systolic and diastolic pressures were taken under the standard conditions of exercise used with Case 1 (see Figure 7). The most striking result was a fall in resting pulse rate. Systolic and diastolic pressures increased slightly at the same time that the pulse rate decreased. The tongue remained smooth and red throughout administration of vitamin B<sub>1</sub>. One week following the addition of yeast, regeneration of papillae was evident and at the end of two weeks the tongue was entirely normal in appearance. Flu-

oroscopic examination of the gastro-intestinal tract during the control period showed the same marked, irregular filling defect of the stomach noted in 1924 and believed to be due to gastric polyposis. The stomach was hypertonic; peri-

implication is clear that definite changes in either the absorption or utilization of food-stuff must have taken place. The fact that serum protein increased during vitamin therapy although the protein intake remained constant may be attrib-

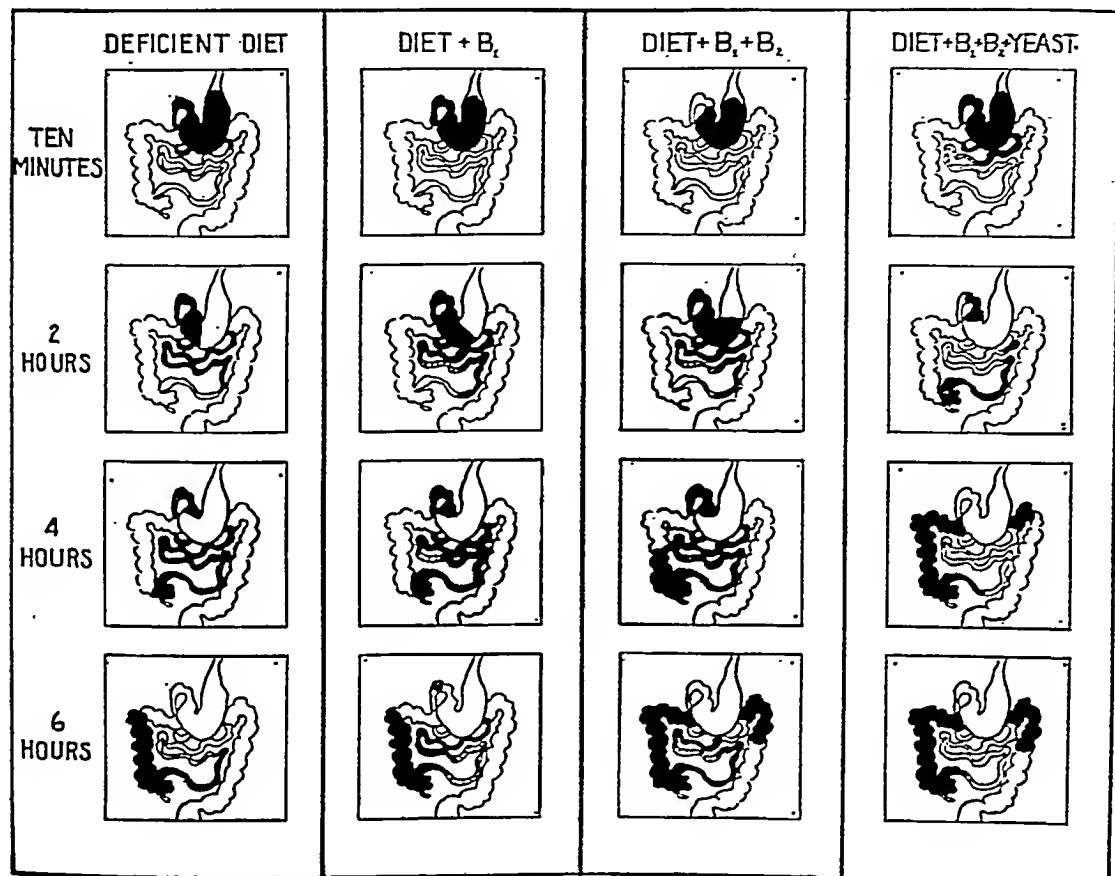


FIG. 5. CASE 1. DIAGRAMMATIC REPRESENTATION OF ROENTGEN APPEARANCE OF THE GASTRO-INTESTINAL TRACT DURING THE DIFFERENT EXPERIMENTAL PERIODS, 10 MINUTES, 2, 4 AND 6 HOURS AFTER A BARIUM MEAL.

stalsis was active and produced deep segmentation. The stomach emptied quickly and the opaque medium progressed very rapidly through both small and large intestines. There was no visible change in gastro-intestinal tone or motility after 3 months on combined vitamin therapy.

#### DISCUSSION

The observed increase in weight, one of the most striking results of vitamin therapy, deserves comment. It occurred, associated with positive nitrogen balance, on a constant diet and in the face of loss of edema fluid from the body. The

uted to various factors, namely, to increased absorption of protein, increased regeneration of body protein or to redistribution of body water.

It is uniformly found in deficiency experiments in animals that when separate vitamin B fractions are added to the basal diet there occurs a temporary increment in weight which is not maintained until all necessary factors are included in the diet. Similar temporary increase of weight on addition of vitamins B<sub>1</sub> and B<sub>2</sub> was observed in Case 1; but this increase was not maintained until yeast therapy had been begun. Evidence was thus provided that additional factors such as are neces-

sary in animals for maximal gain of weight were likewise required by this patient. That these factors were also necessary for complete relief of symptoms was shown when, upon addition of yeast, symptoms disappeared which had returned toward the end of the period with vitamin B<sub>1</sub> and B<sub>2</sub> combined. Gastro-intestinal symptoms were relieved only after the addition of yeast and it was likewise only at this time that gastro-intestinal tone and motility returned to normal.

explanation of the edema exists. After vitamin therapy both patients became free from edema. No apparent reason for this is provided by our studies. In Case 1, the serum protein had been consistently above the concentration thought to represent the critical level. In Case 2, the edema disappeared before there occurred what is regarded as significant alteration in serum protein concentration. The permeability of the capillary wall to protein measured by an indirect method

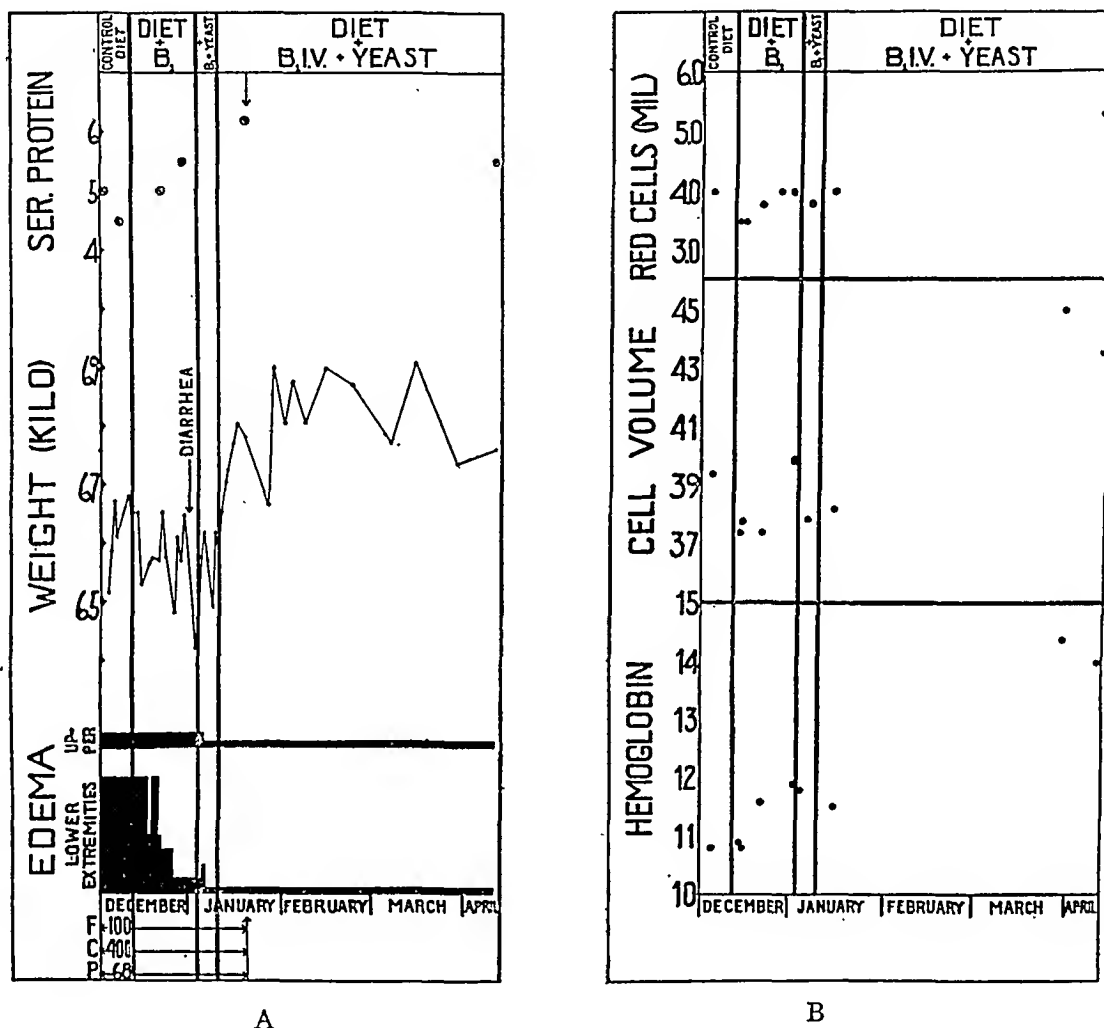


FIG. 6. CASE 2.

A. ALTERATIONS IN EDEMA, WEIGHT AND SERUM PROTEIN ON VITAMIN B DEFICIENT DIET, AND FOLLOWING ADMINISTRATION OF VITAMIN B FRACTIONS.

Daily intake in grams of protein, carbohydrate and fat is recorded at the bottom of the chart. The arrow at the top of the chart indicates the time at which the patient left the hospital.

B. ALTERATIONS IN HEMOGLOBIN, CELL VOLUME AND NUMBER OF RED BLOOD CELLS.

It is of interest that both of the patients studied had definite edema. In Case 2 a ready explanation is at hand in the reduced concentration of serum protein. In Case 1, however, no obvious

was not increased in Case 1. Protein estimation carried out on edema fluid obtained from Case 2 yielded a figure of 0.31 gram per 100 cc. which indicates that here likewise the capillary wall was

not abnormally permeable to protein. It seems extremely unlikely that the observed abnormalities in the cardiovascular system during deficiency, viz., reduction in systolic and diastolic pressures and tachycardia could have been responsible for the edema in the absence of signs of cardiac decompensation and in the presence of normal venous pressure. The fact that capillary pressure

is usually 2 to 3 cm. water above venous pressure (13) permits the assumption that in these patients capillary pressure was not significantly altered and hence that increased filtration pressure was not responsible for the edema. An unusual capacity of the tissues to bind sodium chloride and water has been postulated in cases of "wet" beriberi (14). Whether such an abnormality existed in

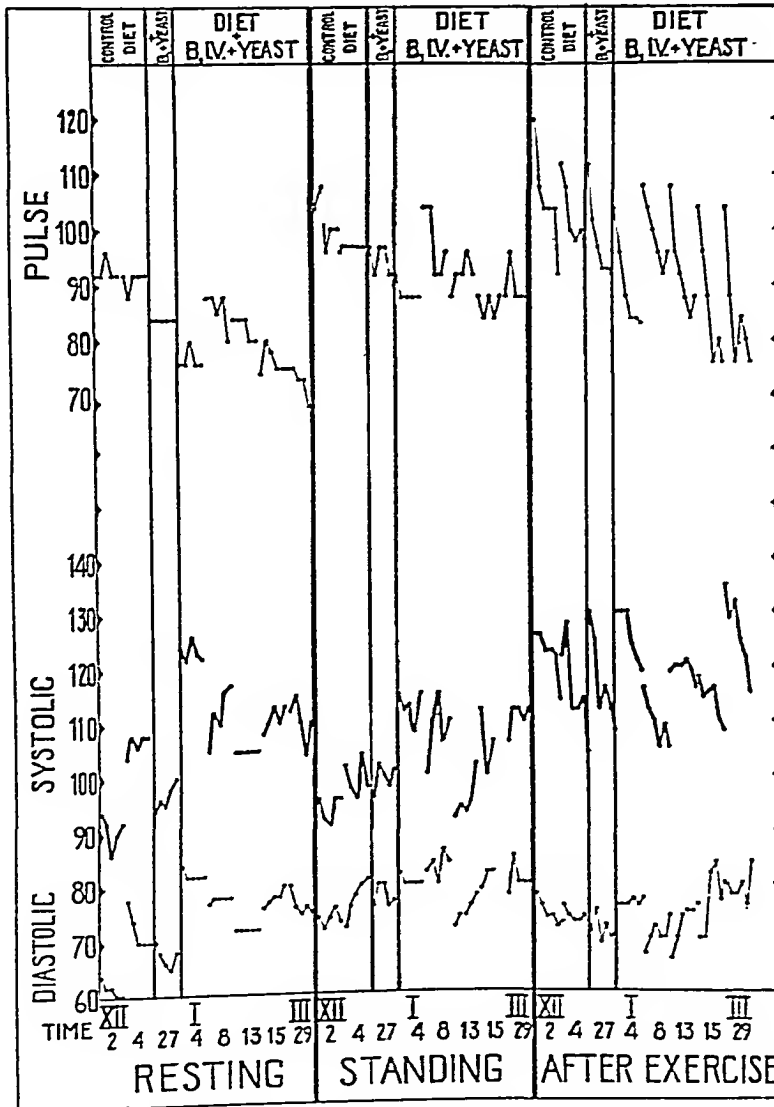


FIG. 7. CASE 2. PULSE, SYSTOLIC AND DIASTOLIC BLOOD PRESSURE, AT REST, AFTER ASSUMING A STANDING POSITION, AND AFTER RAPID ASCENT UP 54 STEPS WHILE ON THE VITAMIN B DEFICIENT DIET AND DURING THE ADMINISTRATION OF VITAMIN B FRACTIONS.

Each point in a continuous line represents successive readings at 1 minute intervals for 5 minutes. An additional reading was made at the end of 10 minutes after exercise.



Case 1 was tested by administering 900 cc. of normal saline solution by mouth and observing the duration of the resultant gain in weight. Such measurements were made both at the height of the edema and at completion of vitamin therapy. Three days were required in both periods for elimination of the fluid. Applicability of this hypothesis to the present situation therefore seems unlikely.

The relationship of vitamin B to anemia is not clear. Strauss and Castle (15) have suggested that some member of the vitamin B complex, after interaction with normal gastric juice may be the active food factor in prevention of pernicious anemia. They and others (16) (17) (18) have obtained remissions using various yeast extracts, autolyzed yeast preparations, egg white and other vitamin B substances. It is still uncertain, however, which vitamin B fraction is the active factor in these responses. Anemia is rarely seen in beriberi (19), and its occurrence is variable in pellagra. There is little evidence that anemia results from complete vitamin B deficiency in rats (20). Recently, however, Rhoads (21) induced a macrocytic anemia in dogs following prolonged restriction of vitamin B<sub>2</sub>. In the present investigation both patients were moderately anemic while in the deficient state. In Case 1 the anemia was of the microcytic type. Following vitamin therapy there was a slow regeneration of hemoglobin and red cells. Anemia in Case 2 was more striking. A tendency toward macrocytosis was observed at the end of the deficient state. At the conclusion of combined vitamin B<sub>1</sub> and yeast therapy this tendency had disappeared. Thus in an individual, Case 1, whose gastro-intestinal function was normal, induced vitamin B deficiency of 6 months duration failed to produce significant anemia. In Case 2, however, who suffered impairment in gastro-intestinal function, vitamin B deficiency was associated with anemia of macrocytic type, which improved following vitamin therapy. It is noteworthy that signs and symptoms of neurological involvement indistinguishable from those of early Addisonian anemia developed in Case 1 during the period of deficient diet and disappeared during vitamin administration. Similar signs were present in Case 2 on admission and likewise showed regression on vitamin therapy.

The fact that disturbances of gastro-intestinal

tone and motility in Case 1 developed during deficiency and disappeared after therapy justifies the conclusion that they are properly attributable to lack of vitamin B. In Case 2, pre-existing gastro-intestinal disease makes difficult the interpretation of effects of vitamin deficiency on the gastro-intestinal tract. There is ample evidence both in animal experiments and in clinical observations of beriberi and pellagra that severe vitamin B deficiency produces disturbances in gastro-intestinal function. Loss of papillae from the tongue (21) and loss of gastric tone while on vitamin B deficient diets (22) have been reported in dogs, as has been delayed gastro-intestinal motility in rats (23).

A sparing action of fat on vitamin B in rats has been demonstrated by Evans and Lepkovsky (24). Conversely, exaggeration of the neuromuscular manifestations of deficient dogs placed on a high carbohydrate diet was reported by Funk (25). In the light of these observations it is interesting that in Case 1, while on a deficient diet, a short period of high fat intake was accompanied by some improvement in symptoms and diminution in edema. When the carbohydrate content of the diet was increased and the fat reduced the improvement ceased.

#### SUMMARY

A patient, who received daily for 5 months a constant quantity of an experimental diet adequate except for moderate limitation of vitamin B, developed signs of deficiency. Clinical signs and laboratory evidence of altered function which developed during deficiency disappeared following addition to the diet of especially prepared vitamin B fractions.

A second patient developed spontaneously similar signs of deficiency which were relieved by similar vitamin B therapy.

The outstanding changes accompanying deficiency were: the appearance of definite symptoms, loss of weight, development of edema, fall in blood pressure, loss of gastro-intestinal tone and motility, signs of neurological involvement and in one patient also an increase in pulse rate and a macrocytic type of anemia.

Vitamin fractions contained in yeast were necessary in addition to preparations of vitamin B<sub>1</sub>

and B<sub>2</sub> for complete relief from the changes which accompanied deficiency.

The controlled observations made upon these two individuals indicate that recognizable clinical signs may develop in otherwise normal persons when the vitamin B content of the diet is only moderately limited. This clinical evidence of deficiency is accompanied by alterations in function, corresponding in many respects to changes described in vitamin B deficient animals.

I wish to acknowledge the kindness of Dr. George Cowgill in calculating the vitamin B<sub>1</sub> content of the experimental diet and in the advice given by him at the outset of this investigation.

I am indebted also to Dr. F. W. Sunderman for the serum protein analyses, to Dr. L. Jonas for the determinations of respiratory quotient, and to Dr. P. H. Shiffer for his assistance in the fluoroscopic examinations of the gastro-intestinal tract.

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# A HISTOLOGICAL STUDY OF THE ARTERIOLES OF THE MUSCLE AND SKIN FROM THE ARM AND LEG IN INDIVIDUALS WITH COARCTATION OF THE AORTA

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There is a form of arteriosclerosis termed diffuse hyperplastic sclerosis which is associated with arterial hypertension. It involves especially the arterioles and smaller arteries and its distribution among the organs is characteristic. It most commonly affects the kidney and spleen, less commonly the pancreas, liver and brain, and rarely, the myocardium, skin, or gastro-intestinal tract. Jores (1), Fahr (2), Evans (3), and Fishberg (4) report that it rarely if ever occurs in voluntary muscle. On the other hand Kernohan, Anderson and Keith (5), in studying the arterioles of voluntary muscle from cases of benign or malignant hypertension, observed pronounced changes which included hypertrophy of the media, intimal proliferation, and reduction in the ratio of lumen to wall. Their observations were confirmed by Barber, Keith and Kernohan (6), and by Pilcher and Schwab (7).

The purpose of this study was to determine whether the pathological changes described by Kernohan *et al.* (5) are found in certain of the arterioles of voluntary muscle from individuals with coarctation of the aorta of the adult type. In this congenital anomaly the bearer suffers hypertension above the stenosis and hypotension below. Therefore, from the skin and muscle of the same individual, one can select for study small arteries of skin and muscle that have been subjected for long periods to quite different blood pressures.

## CLINICAL FINDINGS IN OUR CASES

Five individuals, two male and three female, varying in age from 20 to 33 years, were studied. In four, coarctation of the aorta was the only physical abnormality revealed by careful clinical and laboratory study, while in one there were present in addition a duodenal ulcer and a moderate secondary anemia. Two had slight dyspnea, and one of these had slight numbness of the legs on exercise. Increased arterial pulsations in the neck and markedly decreased arterial pulsations in the lower extremities were findings common to all. There was a loud systolic murmur in each case, best heard over the base of the heart, and well transmitted over the upper thoracic spine; in two instances slight diastolic murmurs also were heard along the upper left sternal border. The aortic second sound was in all cases louder than the pulmonic second. Enlarged and pulsating intercostal arteries in the posterior aspect of the chest were noted in each case. Roentgenological findings were characteristic: the heart was enlarged in three cases and within normal limits in two<sup>1</sup>; there was a fading away or notching of the aortic arch in three; all showed scalloping of the lower rib borders. Electrocardiograms were not remarkable. The average

<sup>1</sup> Lewis (8) has shown that the heart may be definitely hypertrophied in individuals with coarctation of the aorta even though the roentgenological measurements are normal.

TABLE I  
Showing age, sex, and average blood pressures in the 5 cases

	Case I		Case II		Case III		Case IV		Case V	
Age, years.....	29		24		20		28		33	
Sex.....	Female		Male		Female		Male		Female	
Average blood pressure, mm. Hg..	S.*	D.*	S.	D.	S.	D.	S.	D.	S.	D.
Arm.....	183	95	162	100	163	100	177	81	160	87
Leg (calf).....	100	70	85	65	115	65	100	65	95	60

\* S—systolic, D—diastolic.

arterial blood pressures, taken with the patient recumbent, are shown in Table I; the Pachon oscilometer was used for all of the measurements from the leg and some of those from the arm.

While the blood pressures proximal to the coarctation were not very high the increase in pressure in these vessels during exercise or emotion was greater than normal and systolic readings over 220 mm. Hg were usual on such occasions.

It is significant that arteriosclerosis of the retinal arteries was present in two cases and early sclerotic changes in one other. O'Hare and Walker (9) have shown that closely associated with arterial hypertension is retinal arteriosclerosis rather than senile arteriosclerosis of the peripheral arteries, and we believe the hypertension to be causally related to the retinal sclerosis in our cases.

#### METHOD OF STUDY

A block of muscle 1 by 2 cm., with the overlying skin and subcutaneous tissue, was removed for examination from the deltoid and gastrocnemius muscle of each patient. Each specimen was at once divided and one part fixed in formalin and the other part in Zenker's solution. Sections from each piece were stained with eosin methylene blue, Mallory's aniline blue, Mallory's phosphotungstic acid, Van Gieson's and Scharlach R stains. Each section was examined for visible pathological changes in or around the blood vessels. With a micrometer eyepiece, an  $\times 10$  ocular, and an  $\times 90$  objective, the arterioles and smallest arteries, cut in nearly perfect cross-section, were measured. Two diameters of the cross section of the artery bisecting each other at right angles were selected and the ratio between average thickness of lumen and of wall was calculated; the value for the wall was taken as 1. In each case the ratios obtained for the vessels of the skin and muscle of the arm were compared respectively with those of the leg.

An effort was made to establish criteria for the selection of arterioles. Evans (3) used the word arteriole for the smallest arterial twigs with media of 2 or 3 muscle cells in thickness. Kernohan *et al.* (5) used the term for arteries from 25 to 100 microns in outside diameter. Evans (3) has shown that in the kidney the pathological changes in the arterioles differ in

character from those in the arteries from which the arterioles spring. Here at least is an opportunity for distinguishing them based on pathological findings. We measured the afferent arterioles of normal glomeruli, in sections prepared as were those used in this study, and found the great majority of these arterioles had outside diameters between 20 and 30 microns. We have accordingly classified as arterioles those arteries in fixed tissue which are less than 30 microns across while those between 30 and 100 microns across we have classified as "smallest arteries." Allowing 40 per cent for shrinkage in preparation, the arterioles would measure not more than 50 microns under physiological conditions. Obviously, if certain arterioles are thickened by pathological changes some may exceed our arbitrary limit for size; moreover contraction of a small artery during removal and fixation may be sufficient to result in its being classified wrongly as an arteriole. However, definition by measurement will best convey to others the size of artery under consideration.

#### MICROSCOPICAL FINDINGS

The arteries, veins and capillaries of each biopsied tissue were studied microscopically but no pathologically significant changes were found in any. Very occasionally one of the smallest arteries or arterioles showed slight thickening of the intima, slight proliferation of the endothelial cells lining the lumen, and a puckering or undulation of the internal elastic membrane. The slight intimal thickening was characteristically uniform around the circumference of the vessel and the lumen was never greatly encroached upon or narrowed. This appearance was sometimes studied further by tracing the vessel serially into sections differently stained. It was interpreted by us as indicating unequivocally a normal vessel slightly contracted. The proliferation of the lining endothelial cells and the puckering of the internal elastic membrane were associated with and, we thought, most reasonably explained by medial contraction. The degree of this contraction varied among the arteries of similar size in the same section, and could be gauged most readily by the degree of undulation of the internal elastic membrane. These apparent abnormalities were seen in vessels from both skin and muscle and with

TABLE II

*Showing the number of vessels measured, their diameters and ratios of thickness of lumen to thickness of wall*

		Number of vessels measured		Average outside diameter		Extreme ratios lumen to wall		Average ratios lumen to wall	
		Arterioles	Smallest arteries	Arterioles	Smallest arteries	Arterioles	Smallest arteries	Arterioles	Smallest arteries
				<i>microns</i>	<i>microns</i>				
Case I	Muscle of arm	14	9	18.8	45.3	1.55 3.52	1.69 3.37	2.16	2.60
	Muscle of leg	7	2	20.3	38.2	1.70 2.80	1.81 2.18	2.20	1.99
	Skin of arm	1		10.0		2.18		2.18	
	Skin of leg	21	1	19.1	27.6	1.44 3.11	2.18	2.21	2.18
Case II	Muscle of arm	6	5	20.1	37.2	1.57 2.27	2.05 2.66	1.80	2.43
	Muscle of leg	5	3	22.3	49.2	1.42 2.60	1.87 3.05	2.23	2.52
	Skin of arm	4	5	21.0	48.1	1.83 2.26	2.25 3.31	2.02	2.59
	Skin of leg	2		16.2		2.26 2.49		2.37	
Case III	Muscle of arm	9	3	19.5	59.2	1.52 2.56	2.40 3.70	2.07	3.07
	Muscle of leg	3	5	18.4	46.3	1.25 2.28	1.15 3.21	1.84	2.41
	Skin of arm	9	2	16.3	59.6	1.58 2.33	2.31 2.58	1.96	2.44
	Skin of leg	5	1	20.1	39.2	1.75 2.25	2.00	2.01	2.00
Case IV	Muscle of arm	15	2	19.0	33.8	1.91 3.05	1.71 3.00	2.46	2.35
	Muscle of leg	15	1	18.2	87.2	1.46 2.34	2.04	2.00	2.04
	Skin of arm	7		18.0		1.43 2.33		2.02	
	Skin of leg	13		19.5		1.36 2.82		2.60	
Case V	Muscle of arm	8	2	17.7	93.4	1.61 2.62	2.72 3.37	2.13	3.03
	Muscle of leg	5	3	17.0	64.4	1.33 2.19	2.00 3.03	1.85	2.62
	Skin of arm	8		17.8		1.61 2.38		2.08	
	Skin of leg	9		16.7		1.70 2.70		2.14	

TABLE III

The findings in Table II summarized and arranged for easy comparison of the determinations made from the vessels of arm and leg

	Muscle				Skin			
	Arterioles		Smallest arteries		Arterioles		Smallest arteries	
	Arm	Leg	Arm	Leg	Arm	Leg	Arm	Leg
Average outside diameter in microns	19.0	19.2	53.7	57.1	16.6	18.3	53.8	38.4
Extreme ratios lumen to wall	1.52	1.25	1.69	1.15	1.43	1.36	2.25	2.00
	3.52	2.86	3.70	3.21	2.38	3.11	3.31	2.18
Average ratios lumen to wall	2.12	2.02	2.69	2.32	2.05	2.26	2.51	2.09

similar frequency in tissues from either the arm or leg. They were considered not pathologically significant.

The ratios for thickness of lumen to thickness of wall were calculated for the arterioles and for the smallest arteries, and those for the vessels of the skin and muscle of the arm were compared, respectively, with those of the leg. These results are given in Tables II and III.

Table II gives the number of arterioles and smallest arteries measured in each tissue, their average diameter, and the extreme and average ratios for thickness of lumen to thickness of wall. While there was considerable variation in these ratios for the arterioles (1.25 to 3.52), the great majority had a ratio approximating 2. The average ratios for arteriolar lumen to wall ranged from 1.80 to 2.60. The ratios for "smallest arteries" ranged from 1.15 to 3.70 but the great majority had ratios somewhat greater than 2. The average ratios for the "smallest arteries" ranged from 1.99 to 3.07.

Table III is a summary of Table II and gives the averages of the separate values for the 5 cases. It shows clearly that the vessels of the arm and leg were similar with respect to their ratios for thickness of lumen to thickness of wall. The average ratio for the arterioles of the arm muscle was 2.12 and for the leg 2.02; the corresponding ratios for the smallest arteries were slightly greater, 2.69 for the arm and 2.32 for the leg.

The above data taken collectively confirmed the visual impression that the vessels studied were normal; the muscle cells were neither wasted nor hypertrophied and the nuclei were distinct; there was no hyperplasia or splitting of the elastic lam-

ina; the intima and the lining endothelial cells were not considered abnormal. There was no hyaline or fatty degeneration.

#### DISCUSSION

The increased blood pressure proximal to the coarctation of the aorta in these 5 cases is explicable only on mechanical grounds. While this increase was not very great it was prolonged over a long time and was accompanied by cardiac enlargement in at least three instances and definite retinal arteriosclerosis in two. Distal to the coarctation the blood pressure was conspicuously low and the arteries were not subjected to any strain. Despite this difference in blood pressure, however, the small vessels of the skin and muscle of the arm were similar histologically to those in the leg, and our examination failed to discover any changes pathologically significant.

Our cases differ from those of Kernohan *et al.* (5) in several respects. First, they belong to a younger age group; it is possible that a similar strain on the arterioles of older individuals would produce pathological changes. Secondly, the degree of hypertension was not so great; a more severe hypertension even though of shorter duration might be more destructive. Thirdly, the hypertension was of known origin in our cases and limited to the upper part of the body whereas in cases with benign or malignant hypertension where the cause remains unknown there may perhaps be some noxious factor damaging the arterioles. Nevertheless our negative findings are of considerable importance in that they rule out slight to moderate degrees of hypertension maintained over long intervals of time as a cause of

pathological changes in the smallest arteries and arterioles of the voluntary muscle and skin of the arm in young individuals.

#### SUMMARY

We have found that the hypertension associated with coarctation of the aorta in 5 young individuals has not caused sclerosis of the arterioles or smallest arteries of the skin or voluntary muscle. In these 5 cases the systolic blood pressure in the arm averaged 70 millimeters more than that in the leg and the diastolic pressure in the arm 28 millimeters more than that in the leg. Nevertheless, it was impossible by biopsy in these cases to distinguish the arterioles and smallest arteries of the arm from those of the leg.

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# THE METABOLIC MEASUREMENT OF THE WATER EXCHANGE<sup>1</sup>

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Water is provided to the body in the form of obvious fluids, the water contained in solid foods, and the water produced in the oxidation of foodstuffs in the body; it is dissipated as vapor through the skin and lungs, and as liquid through the kidneys, bowel and skin. The common practice of attempting to estimate water balances by comparing the volume of the urine with the obvious fluids ingested is, therefore, quite inaccurate. The water of the food, drink, urine and feces may be determined directly. The water derived from the oxidation of foodstuffs and that lost through the skin and lungs can not be so simply measured. An indirect method for the estimation of these fractions of the water exchange has, therefore, been proposed by Newburgh (1). Estimations of water exchange by this method have yielded improbable results in many of our cases. Failure to obtain good results can not be attributed to disregard of the prescribed conditions, as will be shown below, nor is there reason to doubt the accuracy of the observations. Since this is so, a report of our experiences and a comprehensive investigation of the errors of the method seem warranted.

It has been shown earlier that the calculations of Newburgh may be simplified by the use of the following formula, derived without additional assumptions (2):

$$\Delta W = \Delta \text{Body weight} + (S_e - S_f) + (0.49P + F + C)_v \quad (1)$$

$$\text{Water exchange} = \text{Body weight change} + \text{Solids lost} + \text{Food burned to } (CO_2 + H_2O).$$

Newburgh (1) used, to check the accuracy of his determinations, a so-called "predicted water balance":

$$\text{Predicted } \Delta W = \Delta \text{Body weight} - (P + F)_s \quad (2)$$

$$\text{Predicted balance} = \text{Body weight change} - \text{Foodstuffs stored.}$$

<sup>1</sup>Part of the expense of this investigation was defrayed by a grant from the Ella Sachs Plotz Foundation.

As the fat metabolized is estimated in the same manner for both predicted and determined balances, one can hardly serve as a check on the other in this respect. Furthermore, since "predicted balance" neglects entirely changes in the salt content of the body and in the quantity of unassimilable materials in the gastro-intestinal tract, it can equal determined balance only when the intake of salt and roughage equals the output and when no fat is lost in the stools. Actually in the experiments of Newburgh (1) the predicted balance falls short of the determined balance in every case except one. The discrepancies are in the direction which is to be expected, since "predicted balance" neglects stool fat. The greatest discrepancies occurred on days when unusually large stools were passed. This again accords with expectation, since on such days the excretion of roughage is likely to exceed the intake, causing the predicted value to fall even further below the determined value. It is evident that the "predicted balance" can not be used to verify the "determined balance."

There can be no doubt that Equation 1 represents a true expression of water exchange. It remains to evaluate the accuracy of the various measurements and assumptions involved. These are: (a) accurate measurements of the solids of ingesta and excreta; (b) the assumption that protein burned can be estimated accurately from nitrogen excretion; (c) the assumption that carbohydrate fed is identical with carbohydrate burned; (d) accurate measurements of the foodstuffs fed; (e) a method for the determination of total metabolism, from which fat metabolized can be estimated.

(a) *The determination of the solids of ingesta and excreta* is not an extremely accurate procedure. So great are the difficulties in drying such materials completely without loss by volatilization or decomposition that accuracy greater than  $\pm 5$  per cent should not be expected. Since the total



solids of urine and feces are only about 50 grams daily, errors on the excretory side of the balance would be negligible. The solids of the ordinary diet for adults, however, are of the magnitude of 500 grams daily, and errors in this measurement are quite significant. To obtain the weight of food solids, Newburgh (1) dried entire mixed diets, in metal containers, on the steam bath at 70° C. for about three weeks. In one experiment (recorded in Table 9 of the appendix of his report), duplicate diets, in which the total weight of protein, fat and carbohydrate was estimated at 197.5 grams, were so treated for 25 successive days. The extreme values for determined dry weight were 179 grams and 223 grams per day. The average value, 198 grams, allowed for the presence of no salts or unassimilable material. In our experiments food was dried by a different method which proved no more satisfactory. The entire diet was weighed, passed through a meat grinder and mixed in a large container for one hour with a mechanical stirrer. Aliquots of varying weights, in porcelain evaporating dishes, were kept on the steam bath for about 12 hours, in the oven at 95° C. for about 1 hour, and in a desiccator over sulphuric acid until constant weight was reached. Solids so determined frequently fell short of the estimated weight of protein, fat and carbohydrate in the diets, although analyses of samples taken from the top and bottom of the wet mixtures differed by no more than  $\pm 1$  per cent in nitrogen and chloride. Furthermore variations as great as 10 per cent were noted in the determined dry weights of identical diets prepared on different occasions. Newburgh feels that greater accuracy is possible when simple diets are used. The data recorded in the first table of the appendix of his first report (1) do not support this statement. The determined solids of bread are seen to vary from 57.5 to 66.2 per cent; those of milk from 14.2 to 15.5 per cent. These variations are in the neighborhood of 10 per cent. As water exchange is obtained by subtracting from the change of body weight that portion of the ingested solids which is neither excreted nor burned, any incompatibility between determined solids and estimated foodstuffs must introduce an error in the calculation of water exchange.

(b) *The assumption that protein metabolized can be accurately estimated from nitrogen excre-*

*tion.* Over long periods there can be little error in assuming that the nitrogenous end-products of protein metabolism are excreted as soon as they are formed. In short periods this need not be so. This is particularly true when large changes occur in the concentration of urea in the body fluids. In such cases, if the concentrations of urea in the body fluids at the beginning and end of an experiment are known, however, some attempt at correction may be made. Nitrogen excreted as protein in the urine or stool must, of course, be excluded from the calculation since protein excreted as such gives rise to no water and is included in the dry weights of the ingesta and excreta.

(c) *An assumed value for carbohydrate burned.* In prolonged experiments under constant conditions it is assumed, with justification, that carbohydrate fed equals carbohydrate burned. In short experiments, especially in subjects with nutritional disturbances, however, such an assumption is certainly unwarranted and carbohydrate burned can be estimated by no method short of calorimetry. In many conditions in which the analysis of large water exchanges is important, for example in diabetic acidosis or pyloric obstruction, the method of determination falls completely short in this respect.

(d) *For the measurements of the food mixture ingested* dependence must be placed for the most part upon standard dietary tables and certain factors. These tables at best provide only average values, and one has but to glance at them to recognize the extreme variability of common articles of food. Much of this variability probably depends upon the exposure and desiccation to which the foods have been subjected. In the tables of Atwater and Bryant for example (3) the carbohydrate content of raw potato is given as 18.4 per cent, the average of 136 analyses ranging from 13.5 to 27.4 per cent. In certain foods the variability of fat is quite as great. Protein can be directly measured by determination of nitrogen and, while the factor, 6.25, used to convert nitrogen to protein, is not universally applicable, the error involved in its wholesale use is probably relatively unimportant.<sup>2</sup> It must not be over-

<sup>2</sup> It should be mentioned that in this laboratory the nitrogen of mixed diets has been found higher than the predictions of standard tables in most instances. This may be because the foods have not been dried before

looked that factors for the caloric value of all foodstuffs are subject to similar errors. Direct analysis may be employed to obviate these errors and is reasonably satisfactory for extremely simple diets. In many instances, in studies of diseased subjects, however, such diets are unsuitable for application over long periods of time because the patients' tastes must be given some consideration. Analysis of mixed diets is a difficult procedure. Total caloric content determined by bomb calorimetry must be corrected for cellulose content. Furthermore the possibility of significant variations from day to day can not be excluded without frequent analyses.

(e) *Estimation of energy expenditure from insensible perspiration.* Soderstrom and DuBois (4) reported 175 experiments in which total heat production and water of vaporization were determined simultaneously in the Russell-Sage calorimeter on both normal and diseased subjects under carefully standardized conditions. In 27 experiments on 12 normal men, an average of 24 per cent of the total heat produced was expended in the vaporization of water, with extremes of 21 and 28 per cent.<sup>3</sup> When the heat production was increased 10 to 15 per cent by the administration of glucose or protein, water of vaporization increased proportionally. The variability was larger in disease and, in certain conditions, characteristic deviations were observed. In a group of cretins the water loss was small in comparison with the heat production. In patients with heart disease and dyspnea it was abnormally large. This the authors ascribed to increased losses through respiration. In nephritics with dyspnea, however, the loss by vaporization was normal. In typhoid fever, as long as the body temperature remained constant, regardless of its actual level, the normal relation between heat production and water loss was maintained. While the temperature was rising vaporization was deficient; while it was falling there was excessive vaporization. These figures lend strong

support to the general theory that the quantities of water vaporized from the skin and lungs are closely related to the total heat elimination and, therefore, when the body temperature remains constant, to heat production. Nevertheless, it must be recognized that the degree of variability in this relationship amounted to from  $-12$  to  $+15$  per cent even in normal males.

Levine and Wilson (5) in 53 similar experiments with infants found that vaporization of water accounted on the average for 26 per cent of the heat production, with deviations seldom exceeding  $\pm 10$  per cent of the average. Heller and Schwarz (6) have subjected to statistical analysis all the data in the literature from direct calorimetry experiments in which total heat production and water loss have been measured directly. In about 850 experiments, conducted under basal conditions, in which heat production varied from 900 to 2200 calories per day, the proportion of heat eliminated by vaporization approximated closely the average value of 24 per cent found by Soderstrom and DuBois. Above 2200 calories the percentage lost by vaporization was somewhat smaller. From a similar analysis of 400 experiments in which subjects took food but abstained from exercise, Heller and Schwarz concluded that the same relationship obtained when the total heat production varied between 1200 and 2600 calories. Above this the proportion of heat lost by vaporization fell off, just as it had above 2200 calories under basal conditions. This loss of correlation at high levels of heat production may be referable merely to technical errors, failure of the ventilating systems of the calorimeters to prevent the relative humidity of the atmosphere from rising when vapor production was great. This would lead to absorption by the calorimeter and its furnishings of water which would consequently escape measurement.

Benedict and Root (7), in short periods of study under controlled basal conditions, found a relationship between heat production, measured by indirect calorimetry, and insensible perspiration or loss of weight which is defined by the following linear equation:

$$\text{Calories} = 1.325 IL + 19.3 \text{ per hour, (3)}$$

in which  $IL$  represents the insensible weight loss per hour. Jores (8), Heller (9) and others have

analysis and therefore all volatilization of nitrogen has been avoided. That the analyses are correct is attested by recovery experiments and by the fact that nitrogen equilibrium has been secured in prolonged experiments on normal subjects receiving adequate diets.

<sup>3</sup> In one of 2 women studied the deviation from the average was even larger.

confirmed the essential validity of the Benedict and Root equation when applied to subjects under basal conditions.

If 24 per cent of the total heat produced is eliminated by vaporization of water with a heat equivalent of 0.58 calories per gram,

$$\text{Calories} = 0.58IW/0.24 = 2.42IW, \quad (4)$$

$IW$  = Water of vaporization or "insensible water." Furthermore, since the only difference between insensible weight loss and insensible water is referable to exchange of gases,  $IL = IW + (CO_2 - O_2)$ . Under basal conditions the respiratory quotient must be approximately constant, and consequently  $(CO_2 - O_2)$  and therefore  $IL$  must be proportional to heat production. At an average basal respiratory quotient  $(CO_2 - O_2)$  is approximately  $0.1IW$ . Therefore  $IL = 1.1IW$  or

$$IW = 0.91IL.$$

Substituting this value in Equation 4

$$\text{Calories} = (0.91 \times 2.42)IL = 2.2IL. \quad (5)$$

The curve defined by this equation passes through the origin and deviates considerably from that of Benedict and Root.<sup>4</sup> Since the curve of Benedict and Root is evidently incompatible with the relationship between water vaporized and heat production established by calorimetry experiments, it becomes necessary to explain the fact that it conforms with reasonable accuracy to their own data and those of other workers. The curve of Equation 3 crosses that of Equation 5 at a value of 48.5 calories per hour and predictions of metabolism from  $IL$  by Equation 3 do not diverge more than 10 per cent from those by Equation 5 within the limits 66 and 36 calories per hour. Since these include the variations ordinarily encountered in basal determinations in adults, agreement within 10 per cent with the Benedict and Root curve does not prove that this curve is correct. It is hard to escape the conviction that the peculiar course taken by this curve is referable to fortuitous circumstances connected with the limited conditions under which the experiments of Benedict and Root were conducted.

<sup>4</sup> The equation conforms closely to that derived by Levine and Marples (10) from their own data and those of Soderstrom and DuBois,  $\text{Calories} = 2.27IL - 1.25$  per hour.

Johnston and Newburgh (11) used the insensible weight loss as a measure, over long periods of time, of the total metabolism of hospitalized patients who were allowed limited activity. Subsequently Newburgh, Wiley and Lashmet (12), desiring to extend the study to subjects of more varied types under different circumstances of life, abandoned the equation of Benedict and Root, substituting a method of calculation which related heat production directly to insensible water loss. They measured, for periods of 5 to 85 days, the daily insensible weight losses of 8 subjects who were given diets aimed to maintain them as nearly as possible in a constant state of nutrition while they pursued their ordinary activities. This provided data for the estimation of the mixture of foodstuffs burned, the total energy production and the insensible water loss. All the individuals but one eliminated as heat of vaporization 23.8 to 25.2 per cent of the estimated energy production, which varied from 2075 to 3650 calories per day. In the one exception frequent unavoidable labor caused considerable sensible perspiration. To calculate the insensible water loss from the insensible weight loss under varying conditions, they employed a method of approximation. A formula for the direct estimation of fat burned from the determined insensible weight loss, protein burned, and carbohydrate burned, without the use of this series of approximations can be obtained as follows:

$$(a) \quad IL = IW + (CO_2 - O_2), \\ IW \times 0.58 = \text{cal.} \times 0.24,$$

$$(b) \quad IW = 0.414 \text{ cal.},$$

$$(c) \quad (CO_2 - O_2) = (0.42C + 0.14P - 0.07F) \text{ burned},$$

$$(d) \quad \text{Cal.} = (4.1P + 4.1C + 9.3F) \text{ burned}.$$

Substituting (d) in (b), and (b) and (c) in (a)

$$(e) \quad IL = (2.12C + 1.69P + 3.78F) \text{ burned}.$$

Solving for  $F_b$ :

$$F_b = \frac{IL - 2.12C_b - 1.69P_b}{3.78}.$$

This equation has been accepted by Newburgh (13) as a valid condensation and accurate representation of the calculations involved in his method of approximations.

Newburgh, Wiley and Lashmet (12) state that the amount of energy expended in external work in ordinary activities is negligible, becoming significant only when heavy labor is performed. In this case the difference between energy transformations and heat production will become evident only during heavy muscular exercise and heat production can therefore be used as a practical measure of energy production under ordinary circumstances. The technical difficulties encountered in measuring separately heat production and energy expenditure during work have proved so great that the point at which the difference between the two becomes significant has not yet been ascertained. It is hardly to be doubted that there

machine, some effective work must be performed. It has been shown in these experiments, and even more clearly in later experiments of Benedict and Carpenter (15), that, although in the resting subject approximately 23 per cent of the heat eliminated goes into the vaporization of water, with increasing amounts of work the percentage rises far above this level. The data of these experiments are presented in Table I. In some, shown in Table II, it is possible to compare water losses directly measured against insensible weight losses. The agreement is good. It seems clear from these experiments that even during moderate work there may be a significant difference between total energy transformation and heat production

TABLE I

*Relationship of heat of vaporization to heat production and to total energy transformation during varying activity for 24 hour periods. (From Benedict and Carpenter)*

Subject	Rest		Moderate work				Severe work				Very severe work			
	Heat production		Heat production		Energy production		Heat production		Energy production		Heat production		Energy production	
	Total	Vapor	Total	Vapor	Total	Vapor	Total	Vapor	Total	Vapor	Total	Vapor	Total	Vapor
	calories	per cent	calories	per cent	calories	per cent	calories	per cent	calories	per cent	calories	per cent	calories	per cent
E. O. ....	2283	24.7	3662	35.9	3862	34.0								
J. F. S. ...	2133	22.9	3344	29.4	3544	27.8								
J. C. W. ...	2397	21.8					4577	42.8	5147	38.0	7832	55.3	9314	46.4
B. F. D. ...	2213	25.7					4145	40.6	4565	36.8				
A. L. L. ...	2304	24.3					4270	48.6	4727	44.0	6180	56.4	7137	48.8

is such a point and that it requires more exact definition than the terms "heavy labor" or "vigorous muscular exercise." Atwater and Benedict (14) measured total energy transformation by direct calorimetry, and external work by means of a bicycle ergometer, in a group of normal subjects who worked for 8 hours daily on the bicycle. The work is described as reasonable and not at all excessive. In these experiments the average energy transformation exceeded heat production by about 500 calories which was almost the exact heat equivalent of the measured external work. The total energy expenditure was approximately 5000 calories. The measured external work was equivalent to approximately 200,000 kilogrammeters. Moderately active normal subjects may well transform 4000 calories daily, in which case, unless the human body is an extremely inefficient

and that with increasing amounts of work the portion of the total heat eliminated by vaporization increases considerably. It is possible that in Newburgh's experiments exercise was even more moderate and that a slighter increase in the percentage of heat lost by vaporization was balanced by a slight discrepancy between heat production and total energy transformation so that water of vaporization bore the same ratio to total energy production under the circumstances of his experiments that it usually bears to heat production during rest. It is, however, impossible to define the level of activity at which these two processes will neutralize one another. Heller and Schwarz (6), from their analyses of work experiments, conclude that when the heat production exceeds 3000 calories daily the process of vaporization assumes a larger share of the load of heat

dissipation. No distinction is made, in these work experiments, between vaporization from sensible or insensible perspiration. The performance of such amounts of work under ordinary circumstances must entail some sweating. It is important to note that sweat which falls from the body or is absorbed by clothes and bedding, thus escaping vaporization on the surface of the body, has no appreciable value in heat elimination. This factor is minimized in the calorimeter experiments cited, by direct measurement of water vaporized. It is impossible to say, however, that some of this was not vaporized from the floor or furnishings of the calorimeter rather than from the skin, in which case the values for heat elimination by vaporization from the body will be too high.

TABLE II

*Comparisons of insensible water loss (IW) measured directly with insensible weight loss (IL) derived from measurements of body weight changes corrected for weight of ingesta and excreta, during work experiments of Benedict and Carpenter.*

Experiment	IW grams per 24 hours	IL grams per 24 hours
56 .....	4000	4260
57 .....	4100	4600
62 .....	3370	3640
63 .....	3780	3920
64 .....	6050	6640

If the mean temperature of the body is to remain constant, heat production must equal heat elimination. Heat elimination is effected through radiation and vaporization from the respiratory passages and body surface. In the lungs the process of radiation consists of raising the temperature of the inspired air toward the temperature of the body. The amount of heat lost in vaporization should depend on the osmotic pressure<sup>5</sup> of the body fluids and the quantity of water

vaporized. Christie and Loomis (16) have shown that the expired alveolar air is almost, but not completely, saturated with water vapor at the temperature of the body, that it is more nearly saturated after a period of apnea, less during hyperventilation, and that the vapor pressure of the air in the respiratory dead space is much less than that in the alveolar air. Consequently less water is vaporized by shallow respirations than by deep ones. All these factors which determine the amount of water vapor eliminated by the respiratory system presumably have a similar influence upon the heat lost by radiation and upon the respiratory gas exchange. Therefore both the total amount of heat eliminated by the lungs and the fraction referable to vaporization may be expected, on theoretical grounds, to parallel energy production. Benedict and Benedict (17) have shown that under basal conditions approximately 40 per cent of the total water vaporized comes from the respiratory passages.

In the skin the situation is more complex. If water is lost by evaporation from the surface of the body without appreciable quantities of solute, as insensible perspiration, the external layers of the skin must have a vapor tension. The water given off as vapor from these layers to the surrounding air is presumably renewed by diffusion as the osmotic pressure at the surface is increased by the concentrating effect of evaporation. The fluid vaporized in this manner should depend on the surface area of the body and the gradients of temperature and humidity between the surface and the surrounding air.

The quantities of heat lost both by radiation and by vaporization then should be related to heat production, if this is equal to heat elimination, and to the size or surface area of the subject. It is impossible to say, on theoretical grounds, that the effects of surface area and heat production upon radiation and vaporization will be proportional. If mean body temperature is to remain constant, under fixed environmental conditions, radiation and vaporization must be automatically controlled in accordance with total heat production. The temperature of the skin (and possibly of the respiratory passages) must vary with the heat production through the intermediation of the peripheral circulation. Conversely, when the environmental temperature changes, there must be a cor-

<sup>5</sup> It might seem, at first sight, that the heat value of the vaporization of hypotonic sweat would differ from that of the isotonic fluid from which the sweat was derived. However, a certain exchange of heat must have attended the separation of hypotonic sweat from isotonic body fluids. If the sweat emanates from these fluids the heat of secretion plus the heat of vaporization must exactly equal the heat that would be required to vaporize the original isotonic fluid directly, because the energy expended in any chemical or physical transformation is the same, regardless of the methods or intermediate processes by which it is achieved.

responding circulatory adjustment to maintain a relatively constant gradient of temperature between the body surface and the environment. Such adjustments have been demonstrated repeatedly as has also the existence of a temperature gradient between the body surface and the deeper tissues (18).

Apparently, when heat production exceeds a certain maximum or when the gradient of temperature between the environment and the body surface becomes too small, some process of heat elimination more active than the insensible perspiration is required. In the dog, which is unable to sweat, radiation and vaporization through the respiratory passages are increased by panting. In humans the respiratory elimination of heat seems to follow the dictates of oxygen requirements, responding to the temperature control only when heat dissipation by the skin is prevented, for example in hot baths. Instead the sweat glands are called into play to provide a larger volume of fluid for vaporization. There is no special reason for believing, however, that radiation of heat by either lungs or skin must be influenced by the process of sweating. If it is not, the proportion of the total heat loss which is referable to vaporization should increase steadily with the amount of sweat evaporated. That this increase actually occurs during work has already been shown.

Water loss from the skin may occur with or without the intermediation of the sweat glands. Furthermore, it is possible that moderate activity of the sweat glands need not give rise to sensible perspiration. Yet the intervention of the secretory mechanism, even when it is not marked enough to produce sensible perspiration, will presumably increase the proportion of heat dissipated by vaporization. Sweat secretion without sensible perspiration may account for the increase in vaporization noted by Newburgh when a subject experiences a feeling of uncomfortable warmth. That large amounts of water may be vaporized from the skin without the intermediation of the sweat glands has been adequately demonstrated. In subjects with congenital absence of the sweat glands the percentage of heat lost by vaporization is normal unless the metabolism is greatly increased by work, in which case the rise in vaporization is deficient (19, 20). From the washings of the skin of a normal subject who had remained

unbathed for one week during which he experienced no sweating nor sensation of uncomfortable warmth, no appreciable amount of CI was recovered, indicating that the large amount of water vaporized during the week was supplied without the aid of the sweat glands (21). Vasti (22), by an ingenious method for the detection of activity of the sweat glands over small areas, found evidence of activity in only 5 normal subjects at rest, and in these cases the water losses from the same areas were 18 to 72 per cent higher than those observed in the remainder of the subjects. No note is made concerning the presence or absence of sensible sweating in these experiments, although presumably it did not occur. Vasti noted also that in other experiments when the environmental relative humidity was increased without change of environmental temperature vaporization decreased until the relative humidity approached 80 per cent, when it began to increase. At this point the subjects experienced a sensation of uncomfortable warmth. If this sensation of uncomfortable warmth is an indication of sweat gland activity, it may be that the exclusion of experiments in which this occurs, which has been recommended by Newburgh, may eliminate the factor of activity of the sweat glands. Even so, this requirement imposes a marked restriction upon experiments conducted on non-resting subjects since sweating can apparently be induced under certain conditions by changes of humidity as well as environmental temperature. Activity of the sweat glands at high environmental humidity might serve to facilitate evaporation by supplying a solution on the skin surface which has a higher vapor pressure than that of the isotonic fluid of the epidermis.

It is probably true that for normal subjects and with some exceptions for diseased subjects a fairly constant proportion of the heat production is eliminated through the process of vaporization of water under conditions of rest or of slight activity such as dressing and undressing, standing, and walking about the calorimeter, whether or not food is taken, when conditions of temperature and humidity are reasonably constant. It has been noted that the individual variability is of the order of  $\pm 10$  per cent, however, and that certain individuals tend to vary constantly in one direction from the mean. Furthermore when the me-



tabolism is increased by the performance of work, a greater proportion of the heat produced is dissipated by vaporization. It is possible that this occurs only when sweat secretion is called into play.

Heat loss by vaporization bears a fairly constant relationship to total heat elimination during changes of body temperature (23, 24, 25, 26) but heat elimination falls short of heat production during increases of temperature and exceeds heat production when the temperature falls. Therefore, while vaporization parallels heat elimination, it becomes totally unrelated to heat production under these circumstances. If the specific heat of the body is assumed to be 0.83, for a subject of 60 kgm. an increase in body temperature of 1° C. will require that heat production exceed heat elimination by  $60 \times 0.83 = 50$  calories. For studies of 24 hours or more such changes have relatively little significance. For studies over shorter periods, changes of 0.5° C., which may occur even physiologically are significant. With greater changes in body temperature, such as occur in disease, the significance is much greater. For example, during a malarial chill, Barr and DuBois (24) found that the heat production of subject George S. was 153.1 Cal. in 40 minutes while the heat elimination was only 53.7 Cal., the difference serving to raise the rectal temperature 2° C. During this period, 16.7 Cal. were dissipated by vaporization. This is approximately 31 per cent of the total heat eliminated, but only 11 per cent of the heat produced. Temperature changes of considerable magnitude are frequently encountered in disturbances of metabolism without infection, for example, in recovery from diabetic acidosis, during which determinations of insensible losses and water balances have been attempted in this Department. It is apparent that if vaporization is to be used as a measure of heat production in such cases, a correction must be made for change in body temperature. It is also conceivable that in such cases a considerable amount of the heat produced is used to warm the large amounts of fluids administered, so that heat elimination falls even further short of heat production. It would require, for example, 48 calories to raise the temperature of 3 liters of fluid given at 20° C. to the temperature of the body. This might be quite significant in short periods of study.

During the sleeping hours vaporization is greater in proportion to heat production than it is during the waking hours. Thus Benedict and Carpenter (15) found that during the 6 hours from 1 a.m. to 7 a.m., 17 subjects eliminated by vaporization 27 per cent of the total heat produced, while during the waking hours the average for the same subjects was 21 per cent. For 24 hours the average was 23 per cent. The usual fall in body temperature of 0.5° C. during the night and comparable rise during the waking hours could account for the higher percentage of heat eliminated by vaporization during the night. In any event it would be absurd to use the same factor to convert water of vaporization to total heat production in short experiments conducted during the day and during the night.

Unreasonably large variations in the relationship of insensible water to heat production have been frequently encountered in experiments of our own conducted even over long periods. Data from some of these are presented in Table III. The methods employed in these experiments are comparable in accuracy to those of Newburgh. Body weight was determined on a silk balance capable of measuring within  $\pm 5$  grams. The weights of diets measured in the diet kitchen agreed closely with those of duplicate diets weighed in the laboratory. Stools were sent immediately to the laboratory for weighing. Urine was saved at the bedside until the end of a 24 hour period, when it was removed and weighed by the author. The first 4 cases are patients with nephritis, the fifth a patient with diabetes insipidus. In all of these, extremely large variations in the volume of fluids in the body occurred during the course of study. The subjects were asymptomatic and confined to bed at all times. The room temperature was kept at about 20° C. No sensible perspiration or uncomfortable warmth was noted. The experimental diets were used for at least 3 days previous to the beginning of our observations. In spite of this the insensible loss is seen to vary widely during individual periods in each case. Since all the precautions prescribed for the use of the method were observed, these experiments can not be excluded merely because they fail to fulfill expectations.

The last experiment was conducted upon the

TABLE III

*Insensible weight loss (Author's experiments)*

Subject	Dura- tion	Diet	IL	Body weight	Na+K of serum	ILX2.2
	days	calories per 24 hours	grams per 24 hours	kgm.	m.Eq.	calories per 24 hours
J. McC.	2	2630	1702	67.55		3745
	2	2564	1250	67.69		2750
	2	2595	1742	67.34		3832
	2	2568	1554	67.80 68.04		3420
D. C...	2	2455	1832	63.42		4031
	2	2488	1524	64.74		3352
	2	2461	1019	64.88		2241
	2	2464	1717	64.96		3780
	2	2511	1420	64.99 65.23		3122
P. F....	2	2697	1215	62.33		2673
	2	2671	1386	61.66		3050
	2	2673	1224	61.38		2692
	2	2791	1667	61.31		3670
	2	2724	1632	60.11 59.64		3591
J. M...	2	2531	1101	49.50	131	2421
	2	2528	1019	49.99		2241
	2	2508	1310	51.10	125	2881
	2	2450	1134	52.10 54.41	134	2495
M. C...	2	2024	840	73.46	152	1848
	2	2145	1112	74.54	144	2446
	2	2120	1190	76.30	135	2620
	1	2260	1069	74.15	145	2351
	1	2220	1664	74.25	147	3660
	1	2060	851	77.44 74.60	138 145	1872
P. L....	2	3090	1558	74.72		3430
	2		1419	74.64		3120
	2		1477	74.92		3250
	2		1333	74.92		2935
	2		1437	74.36		3161
	2		1265	74.09		2785
	2		1507	74.43		3317
	2		1646	74.60		3621
	2		1529	74.65		3365
	2		1470	74.63 74.40		3235

author who pursued his ordinary activities during the twenty days of the study. During the fourth, fifth and sixth periods he took 40 grams of urea daily, producing a slight water loss as indicated by change of body weight. There were no symptoms referable to the urea ingestion. During these urea periods the average daily insensible loss was far below that of the other days; in fact it was lower than the smallest loss noted on any other day. There is no reason to believe that the urea decreased the energy metabolism to a comparable extent. Certainly in these experiments

any prediction of total metabolism from insensible loss must be erroneous even for 24 or 48 hour periods. Some factor must be present which causes the relation of vaporization to heat production to vary widely from the accepted value.

Much evidence has accumulated to show that changes in the volume of fluid in the body have a profound effect on the proportion of heat lost by vaporization. Manchester, Husted and McQuarrie (27) found that insensible weight loss increased when excessive amounts of fluid were stored under the influence of pituitrin, and decreased during dehydration produced by fluid restriction and ketogenic diets. Newburgh and Johnston (28) have suggested that these results are referable to the fact that the calculations were based on insensible weight losses and not on insensible water losses, and have presented evidence to show that vaporization is uninfluenced by changes in body water of as much as 6 per cent. Levine, Wilson and Kelly (29) were unable to demonstrate that ordinary variations of fluid intake affected the insensible water losses of infants. In dehydrated infants, however, Levine and Wyatt (30) found that insensible losses were distinctly reduced, returning to normal after the volume of fluids in the body had been restored. Jores (31) has found that the insensible loss is abnormally small in states of dehydration and may vary during diuresis or the accumulation of edema. Robert (32) noted striking increases of insensible perspiration when the volume of fluid in his normal subject was expanded under the influence of pituitrin and fluid administration. In our own experiments, although unusually large changes in insensible loss were associated with large changes of the volume of fluid in the body; no correlation between the two could be detected.

The discovery by Gilman and Barbour (33) that insensible water losses are related to the osmotic pressure rather than to the volume of body fluids offers an attractive but not entirely satisfactory solution of the problem. On purely physicochemical grounds it is impossible to explain why a 10 per cent increase of vapor pressure should cause vaporization of water to increase 50 per cent, yet these are the magnitudes of the changes noted by these observers. Such a quantitative discrepancy suggests that the change of vaporization must be provoked by some attendant



physiological reaction. This may possibly have an adaptive purpose. The insensible perspiration is peculiarly fitted for such a purpose since it is apparently able to eliminate water without appreciable amounts of solutes. How vaporization of water and heat loss by radiation can be simultaneously varied in opposite directions by such a physiological reaction it is hard to see, unless certain parts of the mechanism for the dissipation of heat are especially adapted to one or the other of the two functions. If the proportion of the heat lost by vaporization does fluctuate, it follows that the loss by radiation must vary in the opposite direction if the temperature of the body is to remain constant.

If the conflicting evidence presented above is reviewed, it seems quite possible to ascribe the disagreement to differences in the particular methods by which the volume of body fluids was changed in the various experiments. The pituitrin experiments seem particularly important because it has been clearly demonstrated that this hormone causes the retention of water without a proportional amount of salt. The simultaneous administration of pituitrin and water should, therefore, lower the osmotic pressure of the fluids of the body. This is well illustrated by experiments of Robert (32). He gave to a normal 19 year old male, who subsisted on a constant diet containing fixed quantities of fluid and salt, on separate days 1500 cc. of water with and without injections of pituitrin, pitressin or pitocin. After water alone there was a rapid diuresis, unaccompanied by any appreciable reduction of the osmotic pressure of the serum, measured either by cryoscopy or inorganic analysis. The extrarenal loss of weight during the 4 hours which include the diuresis did not differ appreciably from that of the succeeding 4 hours. Pitocin, which modified the renal excretion of water but little, also failed to affect the insensible loss. On the other hand, after pituitrin and pitressin, but little of the water was eliminated in the urine during the first 4 hours, although the excretion of chloride was not greatly modified. Under these circumstances, during the first hours the osmotic pressure of the serum fell and simultaneously the insensible loss of water increased.

These experiments conform to the theory of Gilman and Barbour (33). In other cases, in-

dubitable variations of insensible loss have been reported when, presumably at least, the osmotic pressure of the body fluids remained unchanged. In this category belongs Benedict's (34) study of a subject during a 30 day fast, the relevant data of which are presented in Table IV. In the first

TABLE IV

*Comparison of energy expenditure measured with that estimated from insensible loss in Benedict's fasting man (34)*

Days inclusive	Calories measured	Calories calculated from IW.	(Na + K) urine
			WB* measured
			<i>m.Eg.</i>
			<i>kgm.</i>
1 to 6. ....	9999	13150	144
7 to 12. ....	8745	8780	155
13 to 18. ....	7950	7190	146
19 to 24. ....	7508	7150	137
25 to 31. ....	8808	8800	120

\* Water balance.

2 columns the heat production according to Benedict's own estimates is compared with that calculated from the insensible water loss. The latter was calculated from the body weight changes, urine weights and gas exchange, using Benedict's estimates of the food metabolized for the derivation of the gas exchange. During the first 6 days the heat production estimated from insensible water loss exceeds the measured heat production by more than 30 per cent. Subsequently the agreement between the two methods is reasonably satisfactory. Obviously during the initial diuresis provoked by the fast in this subject, loss of water by vaporization was also accelerated; the ratio of (Na + K) to water loss which is presented in the last column, nevertheless, gives no evidence that the osmotic pressure of the body fluids was reduced by excessive loss of salts during this period. In fact this ratio is higher during the second period when the value for metabolism calculated from insensible loss agrees almost exactly with Benedict's measurement.

In some of our cases (see Table III), it has already been noted that, though the metabolism must have been quite constant from day to day, the insensible loss was so variable that it could hardly be related quantitatively to energy production. No correlation can be discovered between

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the insensible loss and either the volume of body fluids or changes in this volume. For example, in Subject P. F., while the water balance is constantly strongly negative, as evidenced by the rapid loss of weight, the insensible loss in the last 2 periods greatly exceeds that of the earlier periods. That is, the insensible loss increased, although the volume of fluid in the body decreased. In Subject D. C., there is marked storage of water. The largest insensible loss occurs, however, in the first period when the volume of body fluids is smallest. In Subject J. M., retention of water was induced by giving large amounts of fluids, during the first 4 days without salt, during the last 4 days with salt. During the first 4 days, the base of the serum fell about 5 per cent while during the following 4 days the reverse is true. According to Gilman and Barbour, this should result in an increase of insensible loss above the normal during the first 4 days with a corresponding decrease during the last 4 days. Actually the average daily insensible loss of the last 4 days exceeds that of the first 4 days by 162 grams. Furthermore, although the average osmotic pressure of the body fluids during the beginning and end of the experiment must have been considerably higher than during the mid-portion, the average daily insensible loss of the first two days and last two days of the experiment falls short of that of the intervening 4 days by only 47 grams daily. In this experiment the changes in osmotic pressure of the body fluids are probably slowly progressive in contrast with the rapid changes induced by Gilman and Barbour, so that the two are not strictly comparable. The increase in volume of body fluids is approximately twice as great during the last 4 days of the experiment as it is during the first 4 days. The insensible loss is greater during the last 4 days, which is compatible with the findings of Manchester, Husted and McQuarrie. During the last 2 days, however, when the hydration of the body is greatest, the insensible loss is less than during the two preceding days, and is little greater than during the first 2 days when the subject was relatively greatly dehydrated. In Subject M. C., the volume and osmotic pressure of the body fluids were varied greatly by the administration of pituitrin and different amounts of salt. Extreme variability of insensible loss is noted, and although the greatest

insensible loss occurs during the fifth period when the water retention is greatest, the next largest insensible loss occurs during the third period when there is a loss of over 2 kgm. of fluid from the body, and the smallest insensible loss occurs in the first period when there is an increase of over 1 kgm. in the volume of body fluid. With the concentration of base in the serum, and presumably, therefore, the osmotic pressure of the body fluids, decreasing constantly during the first 2 periods, the average daily insensible loss is smaller than it is in the subsequent 2 periods when the base of the serum rises greatly. In this case the variations in insensible loss can be related to changes in neither the volume nor the osmotic pressure of the body fluids.

These experiments not only indicate extreme variability of insensible perspiration, but also afford material to test the predictability of total metabolism from insensible perspiration. In the first 4 experiments, the subjects were maintained on the experimental diet for a considerable period before the experiments were conducted. The diets should have been quite adequate, and in all cases protein was stored. It seems improbable that the wastage of fat indicated by calculation of total metabolism from insensible perspiration in the first three experiments actually occurred under these circumstances. One can only conclude that an unusually large proportion of the heat produced was eliminated by vaporization. In the fourth experiment, the subject of which had nephritis, hypertension and heart disease of rheumatic origin, the insensible perspiration indicates approximate energy equilibrium for the entire study, which is reasonable. The essential difference between the first three experiments in which vaporization was excessive and this one in which the normal relationship between vaporization and total metabolism obtains is not clear. It may be added that in all of these experiments, as well as in similar additional experiments, in which ordinary mixed diets have been employed, it makes little difference whether metabolism is estimated from the measured insensible weight loss, as has been done in Table III, or from insensible water loss calculated indirectly according to the method of Newburgh, Wiley and Lashmet.

That Newburgh's experiments show remarkable agreement between actual and predicted me-

tabolism over long periods of time can not be doubted. Under restricted conditions the insensible perspiration may yield valuable information concerning the energy metabolism, which can be obtained otherwise in no way short of direct calorimetry. It seems extremely doubtful, however, in view of the mass of experimental evidence to the contrary, that the method is applicable during conditions of changing hydration, or of value in the study of edema and diuresis, as has been asserted by Lashmet (35).

#### SUMMARY AND CONCLUSIONS

An attempt has been made to evaluate the errors attendant upon the determination of water balance by the commonly employed method described by Newburgh and his associates. This method attempts, by correcting changes of body weight for changes in the solid constituents of the body, to measure the actual exchange of water. These changes in solid constituents of the body, except under most unusual circumstances, do not exceed 100 grams daily, and usually lie well below this value. It follows that the combined errors of the determinations must be equally small if the method is to possess any practical value. Actually it has been shown that even the determination of dry weights of food and excreta may be subject to quite significant error. The assumption that carbohydrate fed and burned are equal is open to grave objection in experiments under varied conditions of nutrition, and the estimation of carbohydrate fed from food tables is probably subject to an error of at least  $\pm 5$  per cent. Any error in the estimation of carbohydrate burned, however, will be balanced in part by a consequent opposite error in the estimation of fat burned in the formula

$$F_b = \frac{1L - 2.12C_b - 1.69P_b}{3.78}$$

Assuming that the amounts of carbohydrate and protein metabolized can be accurately estimated, the value for fat burned depends upon an accurate prediction of total energy production. For this purpose Newburgh has proposed to make use of the constancy of the relationship between water vaporized from the body and heat production. The error attendant upon such predictions has been shown to be great. The use of an average

value for vaporization might result in any individual in a systematic unidirectional error of 10 per cent in the estimation of metabolism. At a metabolism of 3000 calories per day, this would introduce an error of  $300/9.3 = 32.4$  grams per day into the estimation of fat burned, and thus of water exchange. With the greater variability in this relationship noted in experiments on diseased subjects, a proportionately greater error may occur. The most extreme variability has been observed in experiments in which the volume of fluid in the body is greatly altered, the very experiments which are most interesting from the standpoint of water exchange. It has also been suggested that, contrary to the statement of Newburgh, Wiley and Lashmet, when work is performed, the difference between total energy production and heat elimination need not be insignificant. Furthermore, at least when work is productive of sensible perspiration, the proportion of heat eliminated by vaporization increases greatly over the basal value. The magnitude of the errors incurred in the determination of water exchange by this method are, except in extremely limited conditions, great enough to make it quite impracticable.

At best the method affords no knowledge concerning the allocation of these fluids within the body. It does not distinguish between changes in the volume of fluid in the cells, interstitial fluids or gastro-intestinal tract although changes in these various compartments of the body fluids must have widely differing significance for the body economy.

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# THE INFLUENCE OF IONIZED AIR UPON NORMAL SUBJECTS<sup>1</sup>

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Dessauer and his associates in Germany (1) have published a series of papers on the effects of artificially ionized air on normal and pathological subjects, which have aroused considerable interest among students of ventilation. The results obtained indicate that positive and negative ions produce, respectively, opposite physiological and subjective effects. Positive ions seem to increase the respiratory rate, basal metabolism and blood pressure, and in general produce unpleasant subjective symptoms. Subjects in the Dessauer experiments complained of headache, a sense of fatigue, nausea, dizziness, and general malaise when exposed to these positive ions. Negative ions, on the other hand, decrease the physiological functions mentioned above, and were often accompanied by a feeling of exhilaration and well being.

The most striking results were claimed in the treatment of cases of essential hypertension uncomplicated by renal or cardiac disease. With this group of subjects a considerable reduction of blood pressure after repeated exposure to negative ions was reported. This reduction tends to persist for some time after the cessation of the treatment and the treatment was considered as of fundamental therapeutic importance. The reported data on normal subjects is less extensive, but in general seem inconclusive except with respect to the subjective symptoms.

Küster (6), working in another department of the University at Frankfort, claimed similar effects upon metabolism under practical conditions of ventilation as a result of the use of an air-conditioning system producing smaller negative ions.

The problem of atmospheric ionization in relation to health and ventilation has been dealt with by Yaglou and his associates at Harvard University. In two of the papers of this series (2) (4) extensive data were reported on the physiological

and subjective changes in human subjects after exposure to artificially ionized air (ions of the small molecular type were used). The data seemed to substantiate in part Dessauer's report of the subjective reactions. The physiological findings do not agree with those of the Germans as they show no relation to the sign of the ion used, or, within limits, to the concentration. In the first of these two papers (4) the authors, however, suggest that their data on total metabolism, respiratory rate, pulse rate, and blood pressure before and after ionization indicate a normalizing effect of ionized air (irrespective of polarity) on these physiological functions. In other words, if blood pressure, for example, were high, ions of either sign tended to lower it—if low, they tended to raise it. In the second paper (2) the authors state that "subsequent experiments with improved apparatus have not entirely confirmed our preliminary observations and Dessauer's conclusions"; and that "under the conditions of the present experiments nothing definite was found to justify the use of artificial ionization in general ventilation."

It should be mentioned that the Dessauer studies were made with an apparatus which supplied artificially ionized air (of either polarity) in which the ions consisted largely of charged submicroscopic particles of magnesium oxide dust with a motility range from .007 to .0018 centimeters per second per volt per centimeter. In some instances the entire air of the experimental room was charged, in others the ionized air was delivered through a funnel placed over the subject's face. Ion counts were usually  $10^6$  ions per cubic centimeter or more. Ions in this range of motility reach the lungs in the inspired air and are precipitated there in considerable quantities. The actual amount of transported material, magnesium oxide, is very minute. In the course of a half hour's respiration of ionized air about .33 mgm. of solid material is inhaled, of which about .005 mgm. of charged material is retained (Dessauer (1)).

<sup>1</sup> Contribution No. 3, John B. Pierce Laboratory of Hygiene.

In the studies of Yaglou and his associates the ions used were of the small molecular type having on the average a motility of 1.3 centimeter per volt per centimeter in the breathing zone of the experimental chamber. According to Dessauer ions of this type do not reach the lungs. The external surfaces of the subject, however, are exposed to their action.

The results of the researches mentioned here indicate that the physiological effects of artificially ionized air on normal subjects, if any, are of small degree and subtle in character. Dessauer's published data on normal subjects (using large ions) are scanty and certainly do not indicate any very striking effects. Küster's results are suggestive but may well have been due to some factor other than ionization since what he was comparing was really a room atmosphere with and without air conditioning. Yaglou's last results seem to be negative, but he worked only with small ions which Dessauer believes to be without important influence.

It would certainly seem that rather large ions of the Dessauer type which can penetrate most deeply into the lungs should be in position to produce a maximum effect. It seemed to us, therefore, desirable to apply the Dessauer technique in an intensive study of a group of normal subjects of as nearly comparable physical status as possible (health, age, sex, etc.). Such a study should be thoroughly controlled from a psychological standpoint and sufficiently prolonged to allow of an opportunity for the appearance of cumulative effects. The present program has been undertaken in an effort to close this gap in the literature on the subject.

#### APPARATUS AND METHOD OF ADMINISTRATION

The ion generator used in the present study was identical with that used in the more recent Dessauer studies, and was constructed at the Institute of Experimental Medicine at the University of Frankfurt-am-Main. The apparatus is fully described in the principle Dessauer reference (1). It consists essentially of an elongated glass container through which 1 to 2 liters of filtered air per second are blown. Situated in this container is a small block of pressed magnesium oxide which is heated by a platinum coil to a temperature of about 950° C. Large numbers of ions

of both polarities are given off from this ion-donor to the passing air. Ions of the undesired sign are filtered out as the air stream passes through a wire grid which is charged to a high potential of appropriate polarity. The unipolar air stream is cooled as it passes through a tube in a water bath and delivered through a glass tube to the experimental chamber. The ionic content of the air stream is measured as it leaves the cooling coil with a suitably calibrated silver leaf electroscope. The ionic concentration at this point is from  $1.7$  to  $2.0 \times 10^7$  positive or negative ions, with mobilities in the range of from .007 to .0018 centimeters per second per volt per centimeter. They consist largely of charged submicroscopic particles of magnesium oxide.

The ions were administered to the subjects in our experiments by leading the air stream in a glass tube through a wall into a small metabolism room which adjoined the laboratory in which the generator was set up. Here subjects reclined comfortably on adjustable beds and breathed the ionized air which was directed on to the face by funnels which fitted loosely over the head and assured a high concentration of ions in the breathing zone. Two subjects could be accommodated at a time. The temperature of the metabolism room was maintained at approximately 70° F., with normal indoor humidities.

Ion counts made in the breathing zone showed a concentration of 5 to  $6 \times 10^7$  ions (5,000,000 to 6,000,000) per cubic centimeter. These counts were made with a portable ion counter similar to that used by the Dessauer group (1). After 30 minutes ionization the concentration of ions in the middle of the room was about  $.5 \times 10^7$  (500,000) per cubic centimeter. When the ion generator was turned off this concentration fell in 15 minutes to  $.1 \times 10^7$  (100,000) per cubic centimeter. After 30 minutes the count had a usual value of  $.04$  to  $.07 \times 10^7$  (40,000 to 70,000) per cubic centimeter. The counts were about the same for negative and positive ions. When positive ionization was used the count for negative ions was practically zero, and vice versa.

#### EXPERIMENTAL ROUTINE

Each subject was contracted with for a program of 15 or more separate experiments, each experiment to be performed on a separate day and



the series to be completed in two to three months. Subjects were paid for their services. Each subject was expected twice a week, but it was understood that this routine would be interrupted if minor illness, overwork, or some special indulgence or recreation had occurred on the day or night previous to the experiment. As the subjects were, with one exception, all medical students, the significance of these instructions was appreciated and intelligent cooperation elicited.

Only male subjects were used. They ranged in age from 18 years 8 months to 25 years 8 months. All were in excellent health and had been pronounced normal on recent medical examination. Two subjects had suffered rheumatic attacks 4 to 5 years earlier. Previous to the actual experimentation each subject was put through the entire routine and the data discarded. None of the subjects were told that the experiment was concerned with the effects of ionized air. Most of them learned of it before the experiment was finished. The novelty of the experimental situation had so completely worn off by this time that none of them showed any real concern as to the matter. Each subject spent from 55 to 60 hours in test periods of  $3\frac{1}{2}$  hours at rest and without decided movement or conversation.

The experiments were divided into two groups (1) a morning group under standard basal conditions, and (2) an afternoon group tested after a standard low calorie lunch taken at 12 o'clock at the laboratory and followed by a rest period of two hours after which tests were made.

Six subjects comprised the morning group. They arrived in teams of two at the laboratory at 10 o'clock on the night preceding the morning tests. They slept until 6 a.m. in the comfortably equipped test rooms of the laboratory which were held at a temperature of approximately 60° F. At 6 a.m. they were awakened, allowed to urinate, and transferred to the metabolism room. After 45 minutes the test routine was begun. Thirty minutes before this routine began the motor blower of the ion generator had been turned on in order to adapt the subject to the slight hum of the motor and the sensation of moving air on the face.

The afternoon group consisted of 5 subjects. The subjects took a light breakfast on the day of

the test and arrived at the laboratory at noon. They were given a low calorogenic lunch (Benedict and Benedict (3)) consisting of

1 cup (200 cc.) of caffeine-free coffee,  
16 mgm. of saccharine,  
30 grams of medium cream,  
25 grams of potato chips,

and immediately reclined on the beds in the metabolism room and rested until 2:00 p.m., and after this rest period tests were begun.

No variation whatsoever was permitted from this routine. Subjects arriving late or reporting strenuous activity were dismissed and taken on another date. Under these regulations subjects in both morning and afternoon groups showed minimal variations in the physiological functions measured and there is no doubt that extraneous factors were eliminated to a very high degree.

The following observations were made during each experimental period. Basal or total metabolism (depending upon whether the morning or afternoon group is considered), blood pressure, respiratory rate, pulse rate, oral temperature, and total urine volume. At the end of the experiment subjects were given an opportunity to comment on their subjective state.

The experimental program as a whole was divided, for each subject, into three series of 5 experiments each, which gave a total of 15 separate experimental periods for every subject. In the first series of 5 experiments, designated as the Positive Series, the subject received positive ions during a period of ionization preceded and followed by control periods. The second series of 5 experiments was identical except that negative ions were administered in the middle period. In the third series, no variation was made from the previous routine except that the air stream during the middle period contained no ions.

Each single experimental session of the 15 was thus divided into 3 periods.

*a. First normal.* Thirty minutes before the end of the preliminary rest period the motor blower was turned on and the subject allowed to rest with the air stream blowing on his face. (The air supplied was filtered air from the laboratory—no ions were added.) At the end of this period, oral temperature, pulse rate, blood pres-

sure, and metabolism were taken in the order named.

*b. Test period.* As soon as the last measurement had been taken in the first normal period either positive (Series 1) or negative (Series 2) ions or no ions at all (Series 3) were added to the air stream and the subject allowed to breathe the ionized or un-ionized air for 30 minutes. The routine observations were repeated at the end of this period.

*c. Second normal.* As soon as the measurements in period (b) had been completed the heating current on the ion-donators was shut off and the subject allowed to breathe un-ionized air as in the first normal period. After 30 minutes the observations were repeated as in periods (a) and (b).

Subjects were then questioned as to their subjective state on which they were allowed free comment. At this point, they visited the washroom and reported the total urine volume. This concluded the experiment.

The temperature and velocity of the air stream was carefully regulated so that there would be no cues which might distinguish the ion period from the two normal periods. In like manner the Positive, Negative and Control Series, as groups, were carried out with identical technique. It should be mentioned that the Control Series was divided into 3 periods and treated precisely as if the mid-period had contained ions.

Metabolism was determined by the closed circuit oxygen consumption method using the Benedict-Roth apparatus. Most of the subjects were

familiar with the technique and yielded satisfactory records.

Blood pressure was determined by the auscultatory method using a mercury manometer. The first clear sound was taken as the systolic pressure and the beginning of the fourth phase as the diastolic pressure.

## RESULTS

The only deviation from the program outlined above consisted in 3 series of 4 experiments each rather than 5 each carried out on 2 of the 11 subjects. In all 159 experiments were made, of which 90 were on morning subjects and 69 on afternoon subjects. There was no important difference between the two sets of records except that the steady states for the various measures tended to be slightly higher for the afternoon group. In view of this similarity the results for the two groups have been consolidated.

Three types of controls are incorporated in the data. (1) Normal control periods preceding and following the ion period in each individual test. (2) A complete Control Series duplicating the Positive and the Negative Series. (3) Contrast control, consisting of a comparison of the changes during ion periods in the Positive Series with those occurring during the ion period in the Negative Series.

In Table I are reproduced the complete data for the Positive Series on one subject, Wi. It can readily be seen from this typical case that while variations do occur in individual experiments which are suggestive, they lose their significance

TABLE I  
Complete data on Positive Series. (Subject Wi)

Ex- per- iment num- ber	Date of ex- per- iment	Sub- ject	Series	Room tem- per- ature	Blood pressure			Pulse rate			Respiration rate			Oral temperature		Metabolic rate Total calories per hour			Urine volume
					1	2	3	1	2	3	1	2	3	1	3	1	2	3	
	1934			°F.	mm. Hg	mm. Hg	mm. Hg	per min- ute	per min- ute	per min- ute	per min- ute	per min- ute	per min- ute	°F.	°F.	cal- ories	cal- ories	cal- ories	cc.
90	2/15	Wi	+	71/70	98/68	108/74	112/78	60	66	72	8	8	7	98.2	98.2	76.3	77.9	76.3	430
111	2/22	Wi	+	71/69	104/62	100/68	102/70	60	63	60	10	9	9	97.6	97.8	78.0	72.5	72.5	510
117	3/1	Wi	+	73/75	100/66	104/68	104/64	64	62	66	10	9	6	97.5	97.7	78.1	79.6	79.4	270
127	3/6	Wi	+	69/69	108/66	108/66	106/70	67	64	62	8	9	8	97.7	97.6	78.4	73.9	74.6	490
135	3/8	Wi	+	70/70	98/66	102/68	102/66	63	63	60	13	8	9	97.5	97.6	73.7	73.5	71.5	640



on repeated observation. For example, the basal metabolic rate is increased in the Positive Series in Experiment 90, but decreased in Experiment 111. Repetition of the experiment, however, defines these differences as lying well within the range of normal variability.

It is impractical to reproduce all of the original data here. They can be illustrated, however, by abstracting the most significant variations in the data for individual subjects. In Table III, I have summarized these data on metabolic rate for individual cases. It is assumed that since all of the functions measured show a considerable variability the proper evaluation of such differences as do occur should involve the employment of the accepted statistical techniques for interpreting mean differences between measurements made under control and experimental situations.

TABLE II

*Consolidated results for one subject—N*

(Each figure is the mean of 5 observations and is accompanied by its standard deviation.)

Experimental series	Number of experiments	1st period normal	2d period ions	3d period normal
Blood pressure—Systolic, mm. Hg				
Positive....	5	104 ± 4.1	105 ± 2.6	105 ± 2.6
Negative....	5	100 ± 2.1	100 ± 4.1	102 ± 1.6
Control.....	5	103 ± 3.5	103 ± 4.7	103 ± 3.3
Blood pressure—Diastolic, mm. Hg				
Positive....	5	70 ± 4.2	71 ± 3.9	70 ± 2.2
Negative....	5	67 ± 2.1	62 ± 3.5	68 ± 3.0
Control.....	5	68 ± 3.2	70 ± 4.3	66 ± 3.0
Basal metabolism—total calories per hour				
Positive....	5	59.6 ± 2.3	61.4 ± 4.4	61.7 ± 2.6
Negative....	5	65.4 ± 4.4	61.2 ± 4.2	62.0 ± 3.6
Control.....	5	62.1 ± 1.9	61.2 ± 1.6	61.1 ± 3.3
Respiration rate—per minute				
Positive....	5	11.0 ± .6	10.6 ± .8	11.0 ± 0.
Negative....	5	10.0 ± .6	9.8 ± .8	9.8 ± .4
Control.....	5	9.4 ± 1.2	9.6 ± .8	9.6 ± .5
Pulse rate—per minute				
Positive....	5	53.4 ± 2.2	49.0 ± 2.6	48.6 ± 3.3
Negative....	5	52.6 ± 2.4	50.2 ± 2.6	51.8 ± 3.7
Control.....	5	50.0 ± 1.3	50.4 ± .63	50.8 ± 1.2

The most convenient device for this purpose is the ratio of difference observed between compared means to the standard deviation of the difference (Rugg (5)). This is often called the Critical Ratio. Critical Ratios above 3 are usually accepted as indicating that some constant factor has entered into the values forming the one mean which was not present in the other case. The formula is:

$$\frac{D}{\sqrt{SD^2_{M_1} + SD^2_{M_2}}} = \text{Critical Ratio.}$$

In this formula  $D$  is the difference between the two mean values.  $SD_{M_1}$  is the standard deviation of one mean,  $SD_{M_2}$  the standard deviation of the second mean.

TABLE III

*Maximum change † in metabolic rate (in total calories per hour)*

Subject	Series	Period 1	Maximum change		Critical ratio
			Period		
		<i>mean ± S.D.</i>		<i>mean ± S.D.</i>	
Mo....	—	*58.3 ± 3.0	2	*62.0 ± 2.5	2.06
Sm....	—	68.4 ± 3.6	2	70.5 ± 3.7	.91
K....	+	58.7 ± 2.4	2	63.3 ± 1.3	3.83
N....	—	65.4 ± 4.4	2	62.1 ± 4.2	1.22
P....	+	60.4 ± 2.1	2	64.2 ± 2.8	2.38
W....	C	65.7 ± 3.0	2	68.1 ± 9.7	.52
Li....	+	72.1 ± 3.3	3	67.8	2.69
Wi....	+	77.7 ± .8	2	74.3 ± 2.8	2.61
Mc....	+	72.5 ± 2.6	2	75.3 ± 3.6	1.27
S....	—	74.6 ± 3.2	2	69.5 ± 4.7	1.82
Ha....	—	72.3 ± 4.5	3	79.5 ± 4.0	2.67

\* Each figure is the mean of 5 measurements of metabolism.

† Maximum change—each comparison in this table, and in Tables III, IV, V, VI was obtained from tabulations of the consolidated results of *each* subject. See Table II.

Of 5 positive period changes 3 were increases, 2 decreases.

Of 5 negative period changes 3 were increases, 2 decreases.

Of 1 control period change 1 was an increase, 0 decreases.

In Table III are presented also the Critical Ratios for the greatest difference in metabolic rate between the mean of any first normal period and the mean of any succeeding period for each subject. (The means of the first normal periods were the means of the five first normal periods for each of the three series.) The notation under

Periods indicates where the variation occurs: Example: Subject Mo (1, 2, —) indicates that the greatest change within a series from the mean for the first normals occurred in the mean for the ion periods in the Negative Series. In Subject W (1, 2, C) the greatest change occurs between the first normal mean and 2 period mean in the Control Series. Only one subject showed a change of possible statistical significance, a barely significant increase of metabolic rate, on exposure to positive ions, all others having a Critical Ratio below 3. As shown in the tabulation at the bottom of the table, an analysis of the direction of change in the three series shows no consistency.

TABLE IV

Maximum change in respiratory rate (per minute)

Subject	Series	Period 1	Maximum change		Critical ratio
			Period		
		<i>mean ± S.D.</i>		<i>mean ± S.D.</i>	
Sm....	C	11.4 ± .8	3	12.6 ± .8	2.35
Mo....	C	11.5 ± .8	3	9.6 ± 1.0	3.28
P.....	C	8.2 ± 1.9	2	7.6 ± .8	.64
K.....	C	14.6 ± 1.0	3	15.6 ± .5	2.00
N.....	+	11.0 ± .6	2	10.6 ± .8	.87
W.....	+	9.0 ± 2.1	3	7.0 ± 1.1	.20
Li.....	+	14.5 ± 2.1	3	16.4 ± 1.8	1.46
Wi.....	C	10.2 ± 1.5	3	7.0 ± 1.1	3.95
Mc.....	C	10.5 ± 1.1	2	12.5 ± 1.1	2.53
S.....	+	12.5 ± 1.1	3	10.8 ± .8	2.57
Ha....	—	16.8 ± 1.0	3	22.0 ± 3.0	2.29

Of 1 negative period change, 1 was an increase.

Of 4 positive period changes, 1 was an increase, 3 decreases.

Of 6 control period changes, 3 were increases, 3 decreases.

In Table IV are reported the corresponding data for respiratory rate. Only two subjects Mo and Wi show statistically significant changes between mean values of first normals and succeeding periods. Both these changes, however, occurred between the first and third periods in the Control Series when no ions were used.

In Table V is an analysis of similar data for pulse rate. Two subjects, K and Ha, show statistically significant changes between the mean of the first normals in the positive period and the mean of the second period (positive ions). Note that the changes for the two subjects are in opposite directions. In six instances the largest

TABLE V

Maximum change in pulse rate (per minute)

Subject	Series	Period 1	Maximum change		Critical ratio
			Period		
		<i>mean ± S.D.</i>		<i>mean ± S.D.</i>	
Sm....	+	51.6 ± 1.7	3	55.8 ± 3.9	2.21
Mo....	—	57.0 ± 1.4	3	61.4 ± 4.2	2.20
N.....	+	53.4 ± 2.2	3	48.6 ± 3.3	2.67
K.....	C	65.8 ± 2.8	3	71.6 ± 3.0	3.22
P.....	—	51.6 ± 3.2	3	56.4 ± 3.5	2.29
W.....	+	53.8 ± 3.2	3	55.8 ± 3.2	1.00
Li.....	+	64.0 ± 5.1	3	61.2 ± 5.0	.85
Wi.....	C	65.6 ± 3.5	3	68.2 ± 3.7	1.13
Mc.....	—	66.5 ± 2.6	3	62.2 ± 3.0	2.39
S.....	+	64.0 ± 1.42	3	62.5 ± 2.2	1.25
Ha....	+	72.8 ± 2.6	2	65.0 ± 3.7	3.71

Of 3 negative period changes, 2 were increases, 1 a decrease.

Of 6 positive period changes, 2 were increases, 4 decreases.

Of 2 control period changes, 2 were increases, 0 decreases.

change occurred in the Positive Series, in two instances in the Negative Series, and three times in the Control Series.

TABLE VI

Maximum change in systolic blood pressure (in mm. of Hg)

Subject	Series	Period 1	Maximum change		Critical ratio
			Period		
		<i>mean ± S.D.</i>		<i>mean ± S.D.</i>	
Ha....	+	111 ± 9.8	2	106 ± 2.2	1.11
Mo....	—	96 ± 5.3	3	103 ± 7.4	.39
S.....	—	117 ± 1.0	2	111 ± 4.1	2.86
Sm....	C	114 ± 1.5	3	122 ± 7.7	2.29
Wi.....	C	101 ± 4.1	2	105 ± 2.1	1.90
Mc.....	+	112 ± 2.8	2	107 ± 5.0	1.72
Li.....	C	104 ± 6.6	2	99 ± 2.1	1.61
W.....	—	95 ± 3.7	3	98 ± 2.4	1.50
N.....	—	100 ± 2.2	3	102 ± 1.6	1.67
K.....	C	98 ± 2.4	3	101 ± 2.1	2.14
P.....	—	99 ± 4.4	3	104 ± 3.2	2.03

Of 5 negative period changes, 4 were increases, 1 decrease.

Of 2 positive period changes, 0 were increases, 2 decreases.

Of 4 control period changes, 3 were increases, 1 decrease.

The data of Table VI indicate that no subject showed a change in mean systolic pressure large enough to give a Critical Ratio of 3. Two subjects showed the most significant changes in the

Positive Series, five in the Negative Series, and four in the Control Series.

Table VII gives the corresponding data for diastolic pressure. Four subjects, Ha, Sm, Wi and W, showed changes with Critical Ratios over 3. Two of these changes occurred between periods in the solid Control Series, one between the first and last normal periods in the Positive Series, and one between the first and last periods in the Negative Series. Both of the latter were increases.

TABLE VII

*Maximum change in diastolic blood pressure  
(in mm. of Hg)*

Subject	Series	Period 1	Maximum change		Critical ratio
			Period		
		<i>mean ± S.D.</i>		<i>mean ± S.D.</i>	
Ha....	+	64 ± 6.6	3	74 ± 2.8	3.13
Mo....	—	68 ± 3.5	3	78 ± 4.2	4.00
S.....	+	68 ± 4.8	2	73 ± 4.0	1.61
Sm....	C	76 ± 2.5	3	83 ± 4.0	3.33
Wi....	C	64 ± 1.3	2	71 ± 4.5	3.33
Mc....	C	72 ± 1.7	2	67 ± 3.6	2.50
Li....	—	67 ± 2.1	2	69 ± 1.0	2.00
W.....	—	63 ± 3.0	3	70 ± 3.5	3.33
N.....	—	67 ± 2.1	2	62 ± 3.5	2.78
K.....	C	73 ± 4.5	3	74 ± 2.4	.43
P.....	C	66 ± 2.6	3	70 ± 4.2	1.74

Of 4 negative period changes, 3 were increases, 1 decrease.

Of 2 positive period changes, 2 were increases, 0 decreases.

Of 5 control period changes, 4 were increases, 1 decrease.

This analysis may be summarized with the statement that using the means of five observations on each subject for comparison values, 66 comparisons on each of five variables (metabolism, systolic blood pressure, diastolic blood pressure, respiratory rate, pulse rate) yielded only ten cases (out of a total of  $5 \times 66$ , 330) in which the Critical Ratio of an observed change exceeded 3. Only one subject occurred twice in this total (K). Five of the ten exceeding a Critical Ratio of 3 occurred in the Control Series, four in the Positive Series, and one in the Negative Series. None of these results justify the conclusion that any one of the variables concerned has been consistently affected or that any subject has shown any consistent response to ionization.

The data on oral temperature and urine volume

were entirely negative and are not reproduced here.

#### GROUP RESULTS

The inspection of the individual results makes it clear that no individual case can be regarded as "susceptible" to the effects of ionized air, in so far as the measures used may be accepted as adequate measures of such effects. The group data can only bear this out more clearly.

TABLE VIII

*Metabolic rate (grand averages)  
(in total calories per hour).  
Consolidated results*

Series	Number of subjects	Number of experiments	1st period normal	2d period ions	3d period normal
			<i>mean ± S.D.</i>	<i>mean ± S.D.</i>	<i>mean ± S.D.</i>
Positive...	11	53	<i>a</i> 67.3 ± 6.5	<i>b</i> 68.3 ± 5.5	<i>c</i> 67.8 ± 6.3
Negative ..	11	53	<i>d</i> 67.7 ± 6.0	<i>e</i> 67.2 ± 5.6	<i>f</i> 68.0 ± 6.5
Control....	11	53	<i>g</i> 68.2 ± 5.7	<i>h*</i> 67.9 ± 4.9	<i>i</i> 68.8 ± 5.7

\* No ions.

#### Significance of the difference

Comparison	Difference between means	Critical ratio
1. Cells <i>a</i> to <i>g</i> .....	.9	.34
2. Cells <i>a</i> to <i>b</i> .....	1.0	.83
3. Cells <i>e</i> to <i>i</i> .....	1.6	.66

TABLE IX

*Blood pressure (systolic) (in mm. of Hg).  
Consolidated results*

Series	Number of subjects	Number of experiments	1st period normal	2d period ions	3d period normal
			<i>mean ± S.D.</i>	<i>mean ± S.D.</i>	<i>mean ± S.D.</i>
Positive...	11	53	<i>a</i> 106 ± 7.4	<i>b</i> 105 ± 5.7	<i>c</i> 107 ± 6.0
Negative ..	11	53	<i>d</i> 104 ± 7.3	<i>e</i> 104 ± 5.2	<i>f</i> 105 ± 4.6
Control....	11	53	<i>g</i> 105 ± 8.1	<i>h*</i> 105 ± 7.6	<i>i</i> 106 ± 7.8

\* No ions.

#### Significance of the difference

Comparison	Difference between means	Critical ratio
1. Cells <i>a</i> to <i>d</i> .....	2.0	.64
2. Cells <i>d</i> to <i>f</i> .....	1.0	.39
3. Cells <i>e</i> to <i>c</i> .....	3.0	1.26

TABLE X

Blood pressure (diastolic) (in mm. of Hg).  
Consolidated results

Series	Number of subjects	Number of experiments	1st period normal	2d period ions	3d period normal
			mean $\pm$ S.D.	mean $\pm$ S.D.	mean $\pm$ S.D.
Positive...	11	53	$\overset{a}{67 \pm 5.7}$	$\overset{b}{69 \pm 4.7}$	$\overset{c}{69 \pm 4.2}$
Negative ..	11	53	$\overset{d}{69 \pm 3.5}$	$\overset{e}{69 \pm 3.6}$	$\overset{f}{71 \pm 3.2}$
Control....	11	53	$\overset{g}{69 \pm 4.2}$	$\overset{h^*}{69 \pm 4.2}$	$\overset{i}{71 \pm 4.7}$

\* No ions.

Significance of the difference

Comparison	Difference between mean	Critical ratio
1. Cells <i>a</i> to <i>d</i> .....	.2	.99
2. Cells <i>d</i> to <i>f</i> .....	.2	1.42
3. Cells <i>a</i> to <i>f</i> .....	.4	2.02

TABLE XI

Respiratory rate (per minute). Consolidated results for 11 subjects

Series	Number of subjects	Number of experiments	1st period normal	2d period ions	3d period normal
			mean $\pm$ S.D.	mean $\pm$ S.D.	mean $\pm$ S.D.
Positive...	11	53	$\overset{a}{11.4 \pm 3.1}$	$\overset{b}{11.2 \pm 3.1}$	$\overset{c}{11.3 \pm 3.5}$
Negative ..	11	53	$\overset{d}{11.7 \pm 2.8}$	$\overset{e}{11.3 \pm 3.2}$	$\overset{f}{11.6 \pm 3.3}$
Control....	11	53	$\overset{g}{11.6 \pm 2.6}$	$\overset{h^*}{11.8 \pm 2.8}$	$\overset{i}{11.2 \pm 3.0}$

\* No ions.

Significance of the difference

Comparison	Difference between means	Critical ratio
1. Cells <i>a</i> to <i>d</i> .....	.3	.24
2. Cells <i>g</i> to <i>i</i> .....	.4	.34
3. Cells <i>h</i> to <i>i</i> .....	.6	.51

In Tables VIII to XII inclusive, are summarized the consolidated results on all eleven subjects for each of the physiological measures. For each table the significance of the difference has been computed for three comparisons which have been conveniently designated as Comparison 1, 2 and 3, as follows:

*Comparison 1:* The greatest difference observed between any two of the three "first normal" pe-

riods (in Table VIII this involves cells *a* and *g*)—the three columns of figures in the upper line being designated from left to right as *a*, *b*, *c*, those in the second line as *d*, *e*, *f*, and those in the third line as *g*, *h*, *i*.

TABLE XII

Pulse rate (per minute).  
Consolidated results

Series	Number of subjects	Number of experiments	1st period normal	2d period ions	3d period normal
			mean $\pm$ S.D.	mean $\pm$ S.D.	mean $\pm$ S.D.
Positive...	11	53	$\overset{a}{60.9 \pm 7.1}$	$\overset{b}{59.8 \pm 6.2}$	$\overset{c}{60.7 \pm 6.0}$
Negative ..	11	53	$\overset{d}{60.2 \pm 6.6}$	$\overset{e}{59.6 \pm 6.6}$	$\overset{f}{60.6 \pm 5.9}$
Control....	11	53	$\overset{g}{61.1 \pm 8.1}$	$\overset{h^*}{60.5 \pm 8.0}$	$\overset{i}{62.5 \pm 8.0}$

\* No ions.

Significance of the difference

Comparison	Difference between means	Critical ratio
1. Cells <i>d</i> to <i>g</i> .....	.9	.29
2. Cells <i>g</i> to <i>i</i> .....	1.4	.41
3. Cells <i>e</i> to <i>i</i> .....	3.0	.96

*Comparison 2:* The greatest difference between any "first normal" period and any succeeding period in the same series (in Table VIII, cells *a* and *b*).

*Comparison 3:* The greatest difference between any two of the 9 cells regardless of the series in which it occurs (in Table VIII, cells *e* and *i*).

An inspection of these comparisons for Tables VIII to XII reveals no instance of a reliable difference. In Table VIII (metabolic rate) the direction of the change is suggestive in the Positive and Negative Series, but statistically not significant. Even the suggestion of an opposed effect does not occur in the other tables of this series. It may be noted that the absence of any significant difference between cells *a* and *d*, and *d* and *g* negatives the possibility of any cumulative effect of five successive treatments with ionized air of either sign.

If we forget for the moment Critical Ratios, and tabulate for each variable the observed values according to the series in which they occurred it is found that summing all five variables the greatest variation occurred 19 times in the Control Series, 19 times in the Positive Series, and 17 times

in the Negative Series. The variations are distributed as nearly in the theoretical chance ratio as is possible with integers, a population of 55 and 3 classifications. (In this comparison, group means are used. See Tables VIII to XII.)

### *Normalizing effects*

As was mentioned earlier in this paper Yaglou and his associates (4), using light ions, could find no opposed effects from the use of positive and negative ionized air, but did suggest tentatively, that "ionized air" irrespective of polarity may have a "normalizing" effect upon these physiological functions. It would certainly appear upon inspection of their tables that this is true. In the case of basal metabolism, as an example, initial tests in normal air which fell between 75 and 84 per cent of the normal standard averaged 81 per cent of normal, and on exposure to ionized air showed an average change of +12.7 per cent. Likewise cases falling between 115 and 124 per cent of normal averaged 121 per cent of normal in ordinary air; and on exposure to ionized air showed an average decrease of -9.0 per cent. Tabulations for all variables for both morning and afternoon groups, and for all age groups, showed essentially the same effect.

It seems to us, however, that precisely this effect is to be expected in any distributive arrangement of measurements of this type. Any single

measure, as of metabolic rate, is in a sense, a fallible measure in so far as it deviates from the most probable value under fixed conditions of measurement (namely, the mean of a series of such measurements). Large deviations from a mean value occur less frequently than small deviations, and having occurred, successive measures (the true mean remaining stable) are likely to show either a positive or negative regression toward the mean. If each of 10 subjects shows a basal metabolic rate of 80 per cent of normal (unless there has been a very selective sampling of low basal metabolic rates from the total population) successive tests will usually show a regression of some degree toward the normal of the population.

In this particular instance the critical test of the "normalizing" effect is a comparison with a control series on the same group of subjects.

In Table XIII and Table XIV the data of this study on metabolic rate and blood pressure have been arranged in the distributive manner, both for the consolidated measures of the Positive and Negative Series and for the Control Series. An inspection of these tables demonstrates that the "normalizing" effect is a statistical characteristic of the classification of repeated measures distributed about a mean, and occurs as strikingly in the Control Series as it does in the consolidated ion series.

TABLE XIII

*Metabolic changes during exposure to ionization. (With Control Series)*

Basal observations and observations 2 hours after standard lunch	Heat production in normal air in percentage of DuBois basal standards				
	75-84 (A)	85-94 (B)	95-104 (C)	105-114 (D)	115-124 (E)
Experimental series:					
Average heat production in percentage of DuBois standard.....	80.7	89.8	99.3	107.4	118.7
Average change in ionized air in percentage of DuBois standard.....	+5.0	+2.9	± .0	-3.1	-10.0
Number of observations (11 subjects).....	4	39	47	14	2
Number showing increase (+2 calories or more).....	3	17	14	2	0
Number showing no change (within ±2 calories).....	1	16	15	3	0
Number showing decrease (-2 calories or more).....	0	6	18	9	2
Control series:					
Average heat production in percentage of DuBois standard.....	83.0	91.1	98.5	109.2	
Average change in "ion" period (no ions) during control series in percentage of DuBois standards.....	+6.7	+2.0	-1.4	-3.9	
Number of observations (11 subjects).....	3	17	20	13	
Number showing increase (+2 calories or more).....	2	4	1	3	
Number showing no change (within ±2 calories).....	1	11	13	3	
Number showing decrease (-2 calories or more).....	0	2	6	7	

TABLE XIV

*Change in systolic blood pressure during exposure to ionized air. (With Control Series)*

Consolidated results of morning and afternoon observations	Systolic blood pressure in normal air (mm. Hg)					
	Under 90	90-99	100-109	110-119	120-129	Over 130
Experimental series:						
Average pressure, mm. Hg.....	88.0	95.1	104.0	113.9	121.6	130.0
Average change in ionized air, mm. Hg.....	+6.0	+3.5	+ .2	-3.3	- 3.6	-10.0
Number of observations (11 subjects).....	1	30	45	24	5	1
Number showing increase (+2 mm. or more).....	1	17	10	1	0	0
Number showing no change (within $\pm 2$ mm.).....	0	11	25	10	1	4
Number showing decrease (-2 mm. or more).....	0	2	10	13	4	1
Control series:						
Average pressure, mm. Hg.....		95.0	103.1	113.3	120.0	
Average change in ion period (no ions) in control series, mm. Hg.....		+1.1	-1.3	-2.4	-11.0	
Number of observations (11 subjects).....		18	16	17	2	
Number showing increase (+2 mm. or more).....		4	2	5	0	
Number showing no change (within $\pm 2$ mm.).....		13	10	4	0	
Number showing decrease (-2 mm. or more).....		1	4	8	2	

## SUBJECTIVE SENSATIONS

At the beginning of this study it was planned to make a check list of the subjective reactions reported by Dessauer (1) and Yaglou (2) and to have it filled out by subjects after each experimental period. Early exploratory experiments with subjects not used in this study (Cr, Ro, Hn, Herr) indicated, however, that such a procedure was of doubtful value. It was evident that the check list would measure, in all probability, the subjects' suggestibility more accurately than his psychic reactions, if any, to ionized atmospheres.

With this in mind it was decided to give the subject a chance to express his own impression of his condition. The question was simply, "How did you feel during the experimental period? What comments have you to make?" In Table XV these reactions are classified under the categories built up by the subject's own initiative. There seem to be no good grounds for stating that these reactions reveal anything more than the fact that a three-hour period of complete rest results in drowsiness, and that the continual exposure of the face to an air stream of sensible velocity produces drying of the lips and throat, under conditions of low to medium relative humidity.

Some of the subjects learned of the general nature of the study after the experiment had been under way for some time. None of the subjects at the end of the experiment could distinguish the Control Series from the Positive or Negative Series, nor had they realized that each separate ex-

TABLE XV

*Tabulation of frequency of certain comments made by subjects*

	Positive ions	Negative ions	Control series
	frequency	frequency	frequency
Difficult breathing*.....	5	1	1
Drowsy, sleepy.....	13	12	8
Bad taste in mouth.....	2	0	1
Throat dry.....	3	1	3
Lips dry.....	9	3	6
Watering of the eyes.....	0	4	2
Headache.....	3	3	0
Tired, depressed.....	0	3	3
Face hot.....	1	4	1
Face cool.....	4	2	0
Feel good, stimulated.....	4	4	1
Feel normal.....	14	11	7
No comments.....	19	16	22

\* This item refers to breathing into and out of the metabolism apparatus. Difficulty disappears with practice.

perimental period was divided into sections during one of which they had received ionized air. Two subjects knew that negative ions were "stimulating" and that positive ions were "depressing." These subjects were no more successful than the others in distinguishing the periods, nor did their comments vary in any significant way in the Positive Series and the Control Series. None of the other subjects had any conception of the reported effects of ionized air.

If we consider that the first nine lines in Table XIV represent undesirable sensations and lines 10 and 11 (face cool; feels good, stimulated) repre-

sent desirable sensations, we find the following distribution of opinion:

*Sensations reported per experiment*

	Positive ions	Negative ions	Control series
Undesirable . . . . .	.68	.58	.45
Desirable . . . . .	.15	.11	.01

The slight differences which appear may probably be attributed to the order in which the experiments were performed, both pleasant and unpleasant sensations being sought for by the subjects with more ardor in the first series of tests with positive ions,—an ardor which decreased as routine dulled their interest.

It seems possible that light ions such as were used by Yaglou may produce certain cutaneous sensations (possibly due to the collection of static charges on body hair) which do not occur with the heavy ions used in this study and administered in the manner described.

#### SUMMARY

The experimental group comprised 11 male subjects in normal health with an age range of from 18, 8 months to 25, 8 months. One hundred and fifty-nine experiments each extending over a period of 3½ hours were made. Fifty-three of these were experiments in which positive ions were administered during a 30-minute period preceded and followed by similar control periods. In each of the 3 periods the physiological measurements were repeated. In 53 experiments this routine was repeated with negative ions. In a third series 53 control experiments were made in which the middle or "ionized" air period did not contain ions. Throughout the entire series the apparatus employed for the generation of ionized particles operated as usual with the single exception that ions either were or were not added to the air stream.

The respiration of positive or negative ions (mobility .007 to .0018 cm. per second per volt

per centimeter) in a concentration of 5. to 6.  $\times 10^6$  ions per cubic centimeter of air produces no consistent or reliable effect upon the metabolic rate, systolic or diastolic blood pressure, pulse rate, rate of respiration, oral temperature, or urine volume of healthy, apparently normal young male subjects. Subjective sensations reported by subjects reveal no effects related specifically to the administration of ionized air.

In conclusion it must be said that these experiments do not close the question as to possible cumulative effects of ordinary atmospheric ionization or artificial ionization on health or comfort. Nor do they justify the conclusion that "artificially ionized air" has no effect upon any physiological function or comfort factor. They certainly do tend to justify the opinion that, so far as normal subjects are concerned, such effects are unproven and improbable. As in Yaglou's later experiments, "nothing definite was found to justify the use of artificial ionization in general ventilation." There still remains the question of the reaction of pathological subjects, in particular, cases of essential hypertension, to ionized air. This problem will be dealt with in a later paper from this laboratory.

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# STUDIES OF TOTAL PULMONARY CAPACITY AND ITS SUBDIVISIONS.

## VIII. OBSERVATIONS ON CASES OF PULMONARY FIBROSIS

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The interpretation of the roentgenograph of pulmonary fibrosis in terms of functional respiratory disability is one of the most difficult problems which confronts the physician in industrial medicine. While the anatomical lesions of pulmonary fibrosis and their roentgenographic detection have been studied extensively, the mechanisms responsible for the respiratory disability are inadequately understood. The functional aspect of the fibroses acquires special importance in cases of pneumoconiosis.

Measurements of the total pulmonary capacity and its subdivisions and their relation to anatomical findings in patients with chronic pulmonary diseases have been presented in previous communications (1) (2). The limits of variation in normal subjects, both male and female, and the method for the prediction of the normal capacity of a given subject have been described (1) (2) (3). The purpose of the present communication is to correlate the anatomical findings with measurements of the total pulmonary capacity and its subdivisions in 58 cases of pulmonary fibrosis.

### MATERIAL AND METHODS

Fifty-eight cases with roentgenographic evidence of pulmonary fibrosis have been studied, fifty-seven were male, and one was female (Case 51). The ages of the patients ranged from 31 to 71 years. Sixty-six per cent were below 50 years of age and thirty-one per cent were between 50 and 60 years. Only two cases were over 60 years of age.

All but four cases had been exposed to the inhalation of inorganic dusts from one to forty years. In 45 cases a history of exposure to siliceous dust in various trades (sand blasting, foundry

work and several other mixed occupations) was given by the patients. Eight patients previously had been miners, and one had been a cutter of stone. In the four cases of pulmonary fibrosis with no history of industrial exposure to dust, chronic respiratory infection was the probable etiological factor. Reliable information as to the amount and composition of the dust was not available. Dyspnea, cough, loss of weight and night sweats were the most frequent symptoms. In 35 cases a fairly accurate estimation of the degree of respiratory disability could be made both from the history and from the response to exercise. In all cases dyspnea was experienced on exertion, and in three instances was caused by the slightest activity.

Clinical and electrocardiographic evidence suggestive of myocardial degeneration was occasionally encountered, but only in one instance (Case 56) was a history of cardiac decompensation obtained. There was no evidence of congestive heart failure at the time of the examination. Roentgenographic examination of the lungs, including fluoroscopy, was made in all cases. The changes detected in the roentgenographic films varied from slight accentuation of the linear markings to dense fibrotic lesions, and this has been made the basis for a grouping of our cases. In two cases pulmonary tuberculosis was strongly suspected but could not be definitely established. In 17 cases there was roentgenographic evidence of left ventricular hypertrophy; of these cases twelve occurred in the group of patients with minimal fibrotic changes, increased linear markings. Enlargement of the right ventricle was present in one patient with extensive reticular fibrosis and in one patient with minimal lung lesions; in another one with nodular fibrosis a prominent shadow in the region of the pulmonary

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artery was observed. Electrocardiograms were made in 46 cases. In 18 cases the tracings were normal, while in 16 others there was left axis deviation and of these twelve were in the group of cases with minimal roentgenographic changes. Right ventricular preponderance was found in three cases with more advanced and generalized lesions in the lung fields. In four instances myocardial damage was suspected from the electrocardiograms, and in two other cases a delay in auriculoventricular conduction without dropped beats. Only eight of all the patients examined showed clubbing of the fingers. No correlation existed between the degree of clubbing and the extent of the fibrosis as revealed by roentgenographs. The venous pressure was determined in a few instances by the method of Eyster (4). In seven patients with nodular fibrosis the venous pressure varied between 65 and 110 mm. of water with an average value of 85 mm. In four patients with a history of asthmatic attacks the venous pressure readings were 60, 90, 110 and 180 mm. of water, respectively.

The methods used for the determination of the total pulmonary capacity and its subdivisions, and for the measurement of the chest expansion have been fully presented in the communications already mentioned (1) (2) (3). Briefly summarized they consist of the determination of the residual air by the oxygen dilution method of Christie (5) and of the vital capacity and its components by graphic registration of spirometric tracings. All observations have been made at least in duplicate, and were carried out with the patients in the recumbent position after a preliminary rest of at least 15 minutes. The chest expansion was measured by means of a roentgenograph doubly exposed at a distance of six feet at maximum expiration and inspiration. The product of the area of the lung fields in the position of maximum inspiration and the anteroposterior diameter of the chest in the same position represents the "radiological chest volume." From this volume the corresponding normal total capacity and its subdivisions are predicted. Various writers have assigned different terms and values for the subdivisions of the total pulmonary capacity. We have adopted the terminology described in the first paper of this series. The normal values used

for comparison were obtained from young healthy adults and consequently they may not be strictly applicable to older patients, especially since decreased vital capacity occurs in normal subjects 50 years of age or older (6). The error introduced by using such standards is, however, only relative. Investigations are now in progress, in order to establish normal values for the pulmonary capacity and its subdivisions for older individuals.

#### *Grouping of cases on the basis of roentgenographs of the chest*

One of the main objects of the present investigation was to correlate alterations in pulmonary capacity and its subdivisions with the degree of the pathological change in the lungs. The cases have, therefore, been grouped according to the nature and the extent of the roentgenographic lesions.

*Group I* consists of 23 cases with increased linear markings in the lung fields. Very slight feathering and beading were observed in a few instances.

*Group II* includes the seven cases with a history of chronic bronchial asthma. The roentgenographic findings were similar to those of Group I but in addition the shadow of the diaphragm appeared low and flat and the intercostal spaces were widened.

*Group III.* These 17 patients showed nodular shadows.

*Group IV.* In this group of four cases the nodular shadows show a tendency to agglomerate, giving a mottled appearance.

*Group V.* The four patients in this group presented large dense shadows, chiefly in the upper portions of the lung and in addition showed marked emphysema at the bases of the lungs.

*Group VI.* A fine and diffuse reticular fibrosis involving the entire lung fields was present in the three cases included in this group.

#### *Observations on the pulmonary capacity*

*General findings.* Before considering separately the findings in each of the groups mentioned above, it will be of interest to study the alterations found in cases of pulmonary fibrosis when they are considered as a single group. The results are shown in Table I. The cases with a

TABLE I  
Pulmonary capacity in 50 cases of pulmonary fibrosis \*

	Mean	Standard deviation	Coefficient of variation	Variations
Absolute values				
	liters	liters	per cent	liters
Total Capacity.....	4.61 $\pm$ 0.08	0.91	19.7	2.70-7.06
Vital Capacity.....	2.86 $\pm$ 0.06	0.69	24.1	1.44-4.66
Mid Capacity.....	2.34 $\pm$ 0.08	0.91	38.8	1.26-4.80
Residual Air.....	1.71 $\pm$ 0.05	0.56	32.7	0.68-3.27
Complementary Air.....	2.26 $\pm$ 0.06	0.69	30.4	0.80-4.36
Reserve Air.....	0.63 $\pm$ 0.03	0.28	44.4	0.18-1.64
Relative values (total capacity = 100 per cent)				
Vital Capacity.....	63.0 $\pm$ 0.91	9.6	15.2	39.1-79.6
Mid Capacity.....	50.9 $\pm$ 1.02	10.7	21.0	30.5-81.5
Residual Air.....	37.2 $\pm$ 0.93	9.8	26.3	20.4-60.9
Complementary Air.....	49.3 $\pm$ 1.04	10.9	22.1	18.8-69.5
Reserve Air.....	13.3 $\pm$ 0.64	6.8	51.1	3.2-40.2

Left column represents the mean calculated normal capacity; the right column the mean observed pulmonary capacity. The black area represents the residual air; the line dividing the white area (vital capacity) is the mid capacity level.

\* The cases with a history of bronchial asthma and the single observation in a female patient have been excluded from this summary.

history of bronchial asthma (Group II) and the female patient are not included. The observed mean value of the total capacity was 4.61 liters, which, compared with the calculated normal value of 5.83 liters, indicates a significant decrease in the volume of air in the lungs at maximum inspiration. This decrease in observed total capacity is caused by diminution in the vital capacity, the latter having a mean value of 2.86 liters as compared with the corresponding normal value of 4.55 liters. These changes have been found in all cases investigated. Both components of the vital capacity (the complementary and the reserve air) are equally affected. The observed residual air was moderately increased, being 1.76 liter, as compared with the calculated normal volume of 1.26 liter. The mean observed value of 2.26 liters for the mid capacity corresponded closely with the normal predicted volume of 2.21 liters.

The changes in the absolute values of the pulmonary capacity are reflected in the relative values of the subdivisions (total capacity = 100 per cent). The mean vital capacity for the entire group constitutes 63 per cent of the total capacity as compared with the normal value of 78 per cent. Correspondingly the residual air represents 37 per cent of the total capacity in pulmonary fibro-

sis in contrast with the normal value of 22 per cent. The relative value of the mid capacity is also increased. A graphic representation of the calculated and observed values of the pulmonary capacities and its subdivisions is given in Figure 1. The relative values are presented in Figure 2.

*Findings in the different groups.* A summary of the average findings in the six groups of cases is presented in Table II. A definite correlation exists between average observed values for the pulmonary capacities of the various groups and the nature and extent of the pulmonary lesions seen in the roentgenographic film. The group with minimal changes (Group I) in the roentgenographs, shows the least change in the average values of the pulmonary capacities. The total and vital capacities are moderately reduced and the ratio of residual air to total capacity is at the upper limit of normality. In Groups III and IV in which are included the cases which show nodular shadows, either uniformly or mottled, the alterations in the pulmonary capacities are more accentuated. This is chiefly due to the greater diminution in the vital capacity while the residual volume remains approximately the same. The ratio of residual volume to total capacity is definitely above the normal limits of variation. It is interesting that no significant differences in the values for the

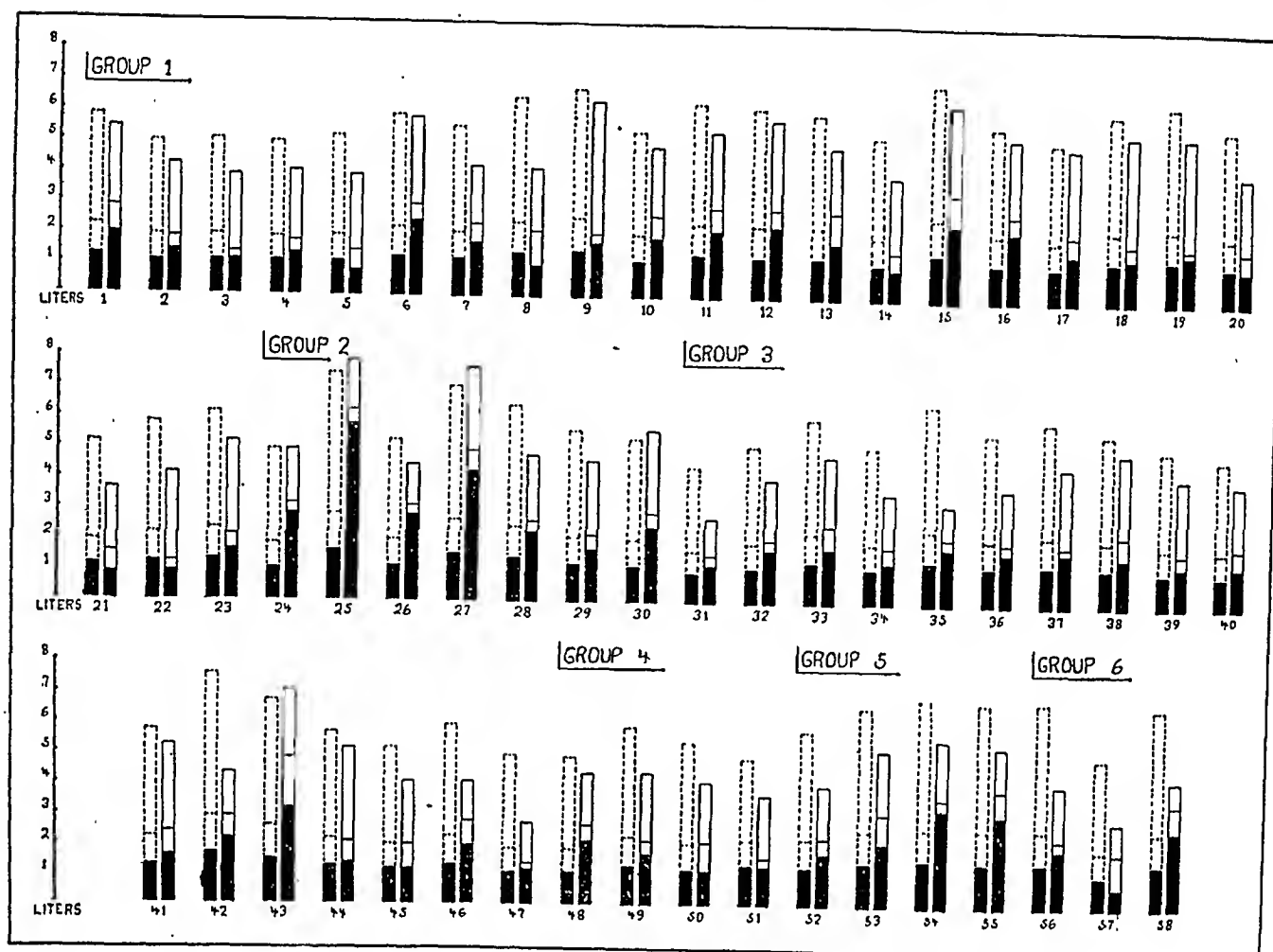


FIG. 1. CALCULATED AND OBSERVED PULMONARY CAPACITY IN 58 CASES OF PULMONARY FIBROSIS.

Each case is represented by two columns. The one on the left is the calculated normal value, and the column to the right is the observed volume. In black, the residual air. The line dividing the white area (vital capacity) is the mid capacity level. The whole column is the total capacity.

pulmonary capacity and its subdivisions exist between Groups III and IV. In Group V characterized by signs of marked emphysema at both bases and large dense shadows in the upper portions of the lungs, the relative values resemble those observed in pulmonary emphysema, except that the total capacity is reduced; although the vital capacity is less than that of the preceding groups, the absolute and relative values for the residual volume are definitely increased. The patients with a diffuse reticular fibrosis, Group VI, show the most marked reduction in vital capacity; the residual volume, however, is only moderately increased. The total capacity is markedly decreased in this group. We have reserved for final consideration the cases with a history of chronic bronchial asthma (Group II). The roentgenographic findings are similar to those observed in Group I except that evidence of pulmonary em-

physema is present. The alterations in the pulmonary capacities are sharply differentiated from the rest of the cases. There is a marked decrease in the vital capacity with a proportional increase in the residual volume so that the total capacity closely approximates the normal value. The value of the ratio of residual air to total capacity is 53.9 per cent, which is abnormally high. These findings are quite similar to those previously described (6) in cases of pulmonary emphysema.

We had the opportunity to observe the changes and the development of bronchial asthma in a patient with pulmonary fibrosis, a Polish man, 51 years of age, a hard coal miner for many years. Before the beginning of asthma the usual changes of pulmonary fibrosis were observed, a decrease in the total and vital capacities with a moderate increase in the residual air (see Figure 3). About a year later this man returned to us with

the history of frequent asthmatic attacks during the interval. A second investigation revealed that the residual air had increased from the previous value of 2.09 liters to 3.80 liters, and that the vital capacity had been reduced about 0.50 liter; the

with minimal pulmonary fibrosis showed a more marked respiratory disability and more accentuated alterations in the pulmonary capacity than that of another patient with nodular type of fibrosis. It is not uncommon to find cases with ad-

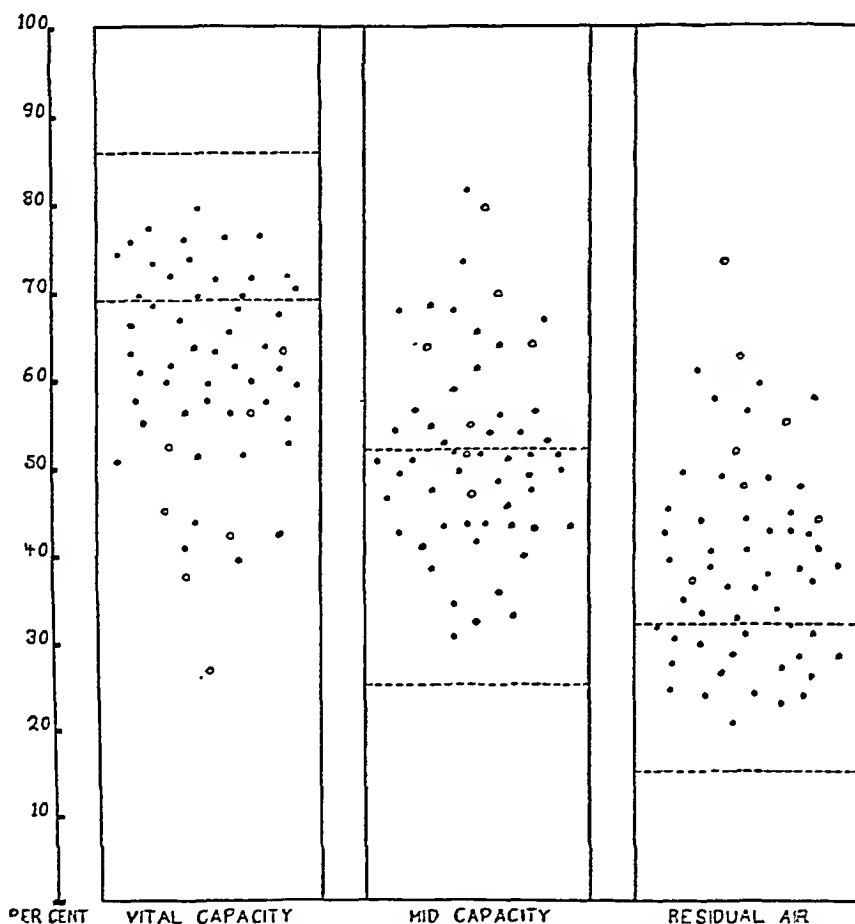


FIG. 2. RELATIVE VALUES OF THE VITAL CAPACITY, MID CAPACITY AND RESIDUAL AIR (TOTAL CAPACITY = 100 PER CENT) IN 58 CASES OF PULMONARY FIBROSIS.

Each dot represents an individual case, those with a history of asthma are represented by circles. Area between interrupted lines is zone of normal variation.

total capacity at this time was greater, 6.40 liters, closely approximating the calculated value of 6.58 liters. In brief, the pulmonary capacity had changed from one typical of pulmonary fibrosis to that usually observed in emphysema.

The fact that the pulmonary capacity may be correlated in a general way with the roentgenographic appearance of the pulmonary lesions must not be interpreted as meaning that the correlation exists in each case. Very frequently a patient

advanced pulmonary lesions with a surprisingly good respiratory adaptation to physical activity. Study of each case is essential. A striking demonstration of this fact is found in those cases with increased residual air (Group II) who show minimal changes on the roentgenographic film, and yet exhibit marked alterations in the pulmonary capacity accompanied by a severe respiratory disability.

TABLE II

*Average observed values of pulmonary capacity and chest expansion and their percentage differences from normal in the different groups of pulmonary fibrosis*

	Group I— 23 cases		Group II— 7 cases		Group III— 17 cases		Group IV— 4 cases		Group V— 4 cases		Group VI— 3 cases	
	Observed values	Percentage difference from normal	Observed values	Percentage difference from normal	Observed values	Percentage difference from normal	Observed values	Percentage difference from normal	Observed values	Percentage difference from normal	Observed values	Percentage difference from normal
<i>Capacities</i>												
Total capacity, <i>liters</i> .....	4.90	-16.0	5.72	-5.4	4.28	-24.5	4.09	-21.9	5.00	-23.4	3.72	-39.6
Vital capacity, <i>liters</i> .....	3.29	-27.6	2.56	-44.8	2.62	-40.3	2.54	-36.4	2.45	-51.4	1.98	-57.4
Mid capacity, <i>liters</i> .....	2.19	-1.4	3.60	+54.4	2.26	+3.6	2.10	+4.1	3.19	+27.7	2.53	+7.4
Residual air, <i>liters</i> .....	1.61	+24.9	3.16	+134.8	1.66	+31.0	1.55	+29.2	2.55	+76.1	1.74	+23.0
Complementary air, <i>liters</i> .....	2.71	-25.0	2.12	-42.1	2.02	-41.4	1.99	-35.7	1.81	-54.9	1.19	-68.4
Reserve air, <i>liters</i> .....	0.58	-37.6	0.44	-55.3	0.60	-34.4	0.55	-38.4	0.64	-40.9	0.55	-14.4
<i>Ratios</i>												
Vital/Total capacity $\times 100$ .....	67.9	-12.9	46.1	-40.9	60.8	-22.0	62.5	-19.9	49.7	-36.3	55.6	-28.7
Mid/Total capacity $\times 100$ .....	44.8	+18.2	61.5	+62.2	52.7	+39.0	50.9	+34.3	63.2	+66.7	67.2	+74.6
Residual/Total capacity $\times 100$ .....	32.1	+45.9	53.9	+145.0	39.2	+78.1	37.5	+70.4	50.3	+128.6	44.4	+101.8
Complementary/Vital capacity $\times 100$ .....	81.9	+3.1	82.8	+4.2	77.2	-2.7	79.0	-0.5	74.1	-6.6	60.0	-24.4
<i>Expansion</i>												
Ratio $\frac{\text{Area at maximum expiration}}{\text{Area at maximum inspiration}} \times 100$	65.6	+5.3	75.4	+21.2	72.5	+16.5	69.8	+12.2	81.1	+30.4	72.5	+16.5
Right diaphragm excursion, <i>cm</i> .....	4.8	-23.8	3.8	-39.7	3.9	-38.1	3.9	-38.1	2.6	-58.7	4.4	-30.1
Left diaphragm excursion, <i>cm</i> .....	5.0	-21.8	4.2	-34.4	4.1	-35.9	4.1	-35.9	2.6	-60.3	3.6	-43.7
Lateral expansion, <i>cm</i> .....	2.3	-28.1	2.0	-37.5	2.1	-34.3	2.5	-21.9	1.4	-56.2	1.7	-46.8
Rib rotation, <i>degrees</i> .....	15.0	-29.9	15.0	-29.9	13.7	-35.9	16.2	-24.3	10.0	-53.2	11.0	-48.6

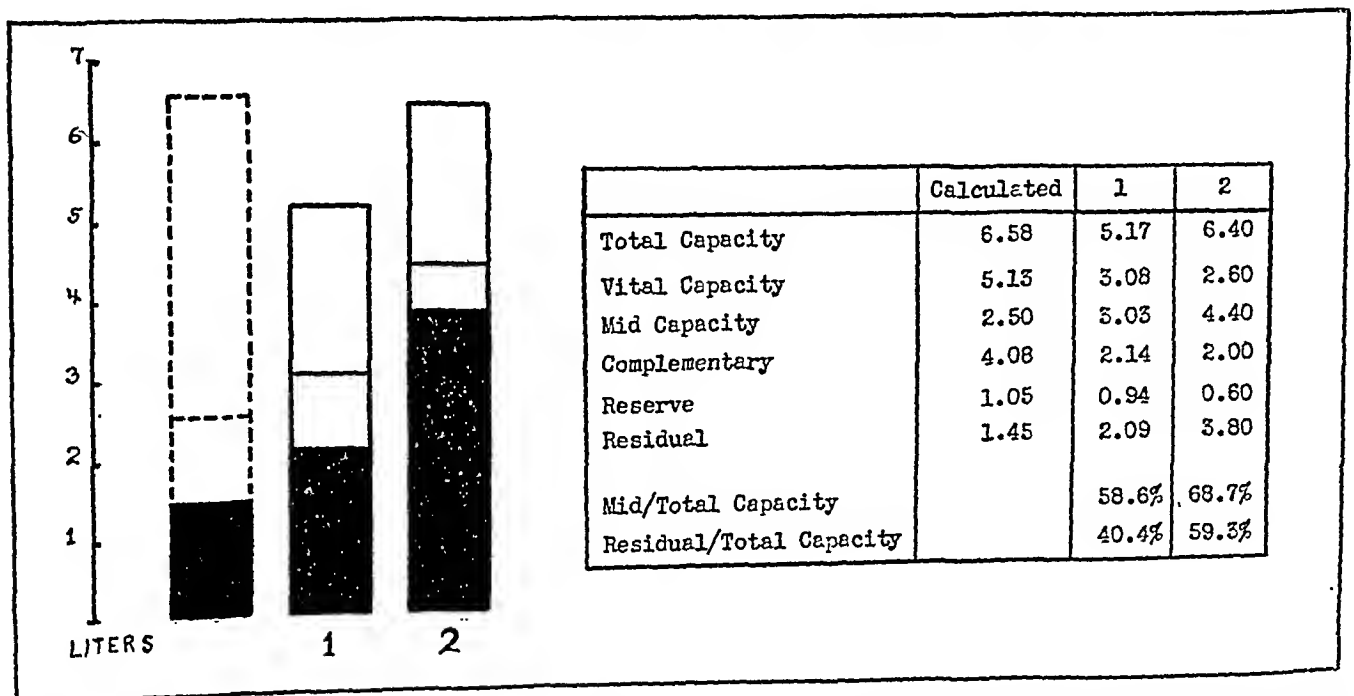


FIG. 3. PULMONARY CAPACITY IN A CASE OF PULMONARY FIBROSIS BEFORE (COLUMN 1) AND A YEAR AFTER THE DEVELOPMENT OF BRONCHIAL ASTHMA (COLUMN 2).

The column at the left with broken lines is the calculated normal capacity.

*Observations on the expansion of the chest*

The different roentgenographic measurements of the expansion of the chest are summarized for all cases in Table VII, and the averages for the different groups are given in Table II. There is a significant decrease in the ability to expand the chest in patients with pulmonary fibrosis. The ratio  $\frac{\text{area at maximum expiration}}{\text{area at maximum inspiration}} \times 100$  has a mean value of 72.0 as compared with 62.0 in normal subjects. The diaphragms have a diminished excursion, the lateral expansion of the chest is reduced and the ribs rotate through a smaller angle in passing from the respiratory position of maximum expiration to inspiration. It is quite interesting that the average changes in chest expansion are also correlated with the nature and extent of the pulmonary lesions in the different groups. Those patients with minimal fibrosis (Group I) are able to expand their chest almost as well as normal subjects but, as the fibrotic lesions become more extensive and diffuse, the expansion of the chest is also correspondingly diminished. The patients of Group II with a history of bronchial asthma and unquestionable emphysema, and those in Group V, exhibit the greatest inability to expand the chest. It must be also emphasized that such average correlation between chest expansion and anatomical lesions,

is not absolutely constant in all cases. Quite frequently a patient with marked anatomical changes shows an almost normal expansion, which again indicates the necessity of studying each case individually.

*Discussion of results from the points of view of probable etiology and occupational history*

It must be clearly emphasized that the consideration of our findings in regard to the pulmonary capacity from these points of view offers considerable difficulties and must be interpreted within certain limitations. The number of cases studied is inadequate to draw any statistical conclusion as to the rôle of each etiological factor, and the history given in many instances was questionable as to accuracy in regard to the exact nature and the length of exposure to dust inhalation. However, some interesting facts have been brought out.

Table III summarizes the average values and the variations of the pulmonary capacity when the cases are grouped according to their occupational history. Of those patients exposed to the inhalation of siliceous dust in various occupations, it is interesting to note that those who had worked in sandblasting gave a history of the shortest period of exposure and the shortest interval between the exposure and the development of the symptoms. They also exhibit the most marked

TABLE III

*Study of 58 cases of pulmonary fibrosis from the point of view of the probable etiology and occupational history*

Occupation	Number of cases	Average duration of			Percentage of cases in the various groups						Pulmonary capacities average values									
		A*	B**	C***	I	II	III	IV	V	VI	Total capacity		Vital capacity		Mid capacity		Residual air		Ratio <u>Residual</u> <u>Total</u> capacity	
											Ob- served values	Differ- ence from normal	Ob- served values	Differ- ence from normal	Ob- served values	Differ- ence from normal	Ob- served values	Differ- ence from normal		
years	per cent						liters	per cent	liters	per cent	liters	per cent	liters	per cent	per cent					
1. Sand blasting.....	10	4.2	3.9	6.0	20.0		60.0	20.0			4.65	-25.9	2.45	-42.4	2.15	+ 4.5	1.51	+33.1	42.1	
2. Iron moulding—Foundry work.....	15	17.3	15.1	22.2	45.6	20.0	20.0	6.6		6.6	5.31	-14.1	3.11	-34.0	2.54	+19.0	2.19	+51.4	29.1	
3. Various mixed occupations (probably exposed to silica dust).....	20	11.2	10.2	13.5	55.0	10.0	33.0		5.0		4.51	-12.2	3.62	-32.7	2.33	+ 8.0	1.51	+42.2	37.1	
4. Coal mining.....	8	14.2	21.5	22.5	25.0	25.0	12.5		37.5		4.41	-22.7	2.42	-42.0	2.47	+11.5	1.59	-51.4	44.0	
5. Stone cutting.....	1	40.0	39.0	41.0			100.0				4.54	-24.5	2.50	-40.5	1.91	-15.5	1.74	-52.9	31.3	
6. Unknown etiology (Infections).....	4				25.0			25.0		50.0	4.55	-25.5	2.55	-40.0	2.20	- 2.5	1.50	-41.1	31.5	

\* A Exposure to dust.

\*\*B Interval between beginning of exposure and initiation of symptoms.

\*\*\*C Interval between beginning of exposure and present investigations.

alterations in the pulmonary capacity and its subdivisions; chiefly affecting the vital capacity which is markedly reduced. A high percentage of the patients in this occupation fall into the groups characterized by nodular fibrosis. Those patients who gave a history of having worked in iron foundries and various mixed occupations had a considerably longer period of exposure and have come to us at a considerably later period. The patients working in these latter occupations belong to the group of cases showing an increase of linear markings in roentgenographs.

### *The respiratory dead space*

The volume of the respiratory dead space is of great significance in relation to the effective alveolar ventilation. An increase in the dead space relative to the tidal air tends toward a less effective alveolar ventilation. In Table IV are presented the results of the calculation of the respiratory dead space from the tidal volume and

TABLE IV

*Respiratory dead space in pulmonary fibrosis*  
(Calculated from the tidal volume and the CO<sub>2</sub> and O<sub>2</sub> percentages of the alveolar and expired air)

Case number	Ventilation per minute	Respirations	Tidal volume	Dead space	
				From CO <sub>2</sub> percentage	From O <sub>2</sub> percentage
	liters	per minute	liters	cc.	cc.
3	10.99	20	0.54	178	195
6	7.59	12	0.62	233	235
7	13.34	20	0.68	307	212
31	8.84	22	0.41	122	90
32	7.36	16	0.48	180	158
33	9.46	22	0.43	216	233
34	6.68	16	0.42	186	193
37	9.66	18	0.52	149	140
48	9.15	20	0.47	133	171
49	10.45	19	0.54	213	248
52	5.06	12	0.42	83	42
57	9.33	14	0.67	195	176
Average	8.99	17.6	0.52	183	174

the CO<sub>2</sub> and O<sub>2</sub> percentages of the alveolar and expired air in twelve cases. The dead space has a value of about 180 cc. which compares with an average tidal volume of 0.52 liter, a figure slightly greater than the average of normal. The results of this calculation are open to objection on the grounds that it is not certain that a true average

sample of alveolar air was obtained in these cases of pulmonary fibrosis. If the values obtained are correct they would indicate that an abnormality of the dead space does not play a significant rôle in the production of respiratory disability in these cases.

We have attempted to reach a more accurate estimation of the dead space based upon the point at which the carbon dioxide concentration assumes a constant value in serial fractional samples of the air of a single expiration. One such curve is shown in Figure 4, in which it may be observed that the flat part of the CO<sub>2</sub> curve seems to be reached before 200 cc. of the air is expired, a figure which is in general agreement with that obtained in the above indirect method of calculation.

### DISCUSSION

The observations presented show that there are evident alterations in the total pulmonary capacity and its subdivisions in pulmonary fibrosis. Apart from a preliminary communication from this clinic (7) there are no previous observations in the literature with which we may compare our findings. Perhaps the most closely related observations have been made in pulmonary tuberculosis. Garvin, Lundsgaard and Van Slyke (8) in 1918 observed a decreased vital capacity and increased residual air in incipient cases, while in more advanced conditions the latter volume was normal, but with a still greater reduction in the vital capacity. These findings were later confirmed by Anthony (9). It appears that a decrease in the total and vital capacities is of most constant occurrence in pulmonary fibrosis, while the residual air is as a rule moderately increased, and may infrequently reach an abnormally high value. Accompanying these alterations there is, in most cases, a decreased ability to expand the chest. These abnormalities show a definite tendency to be correlated with the degree of fibrosis.

Gardner (10) has recently summarized the development of the anatomical changes which result from the inhalation of the inorganic dust particles, and divides the reaction into five general phases: 1—diffuse parenchymatous disease due to the accumulation of phagocytes and local inflammatory changes in the immediately adjacent connective tissue; 2—linear perilymphatic proliferation; 3—

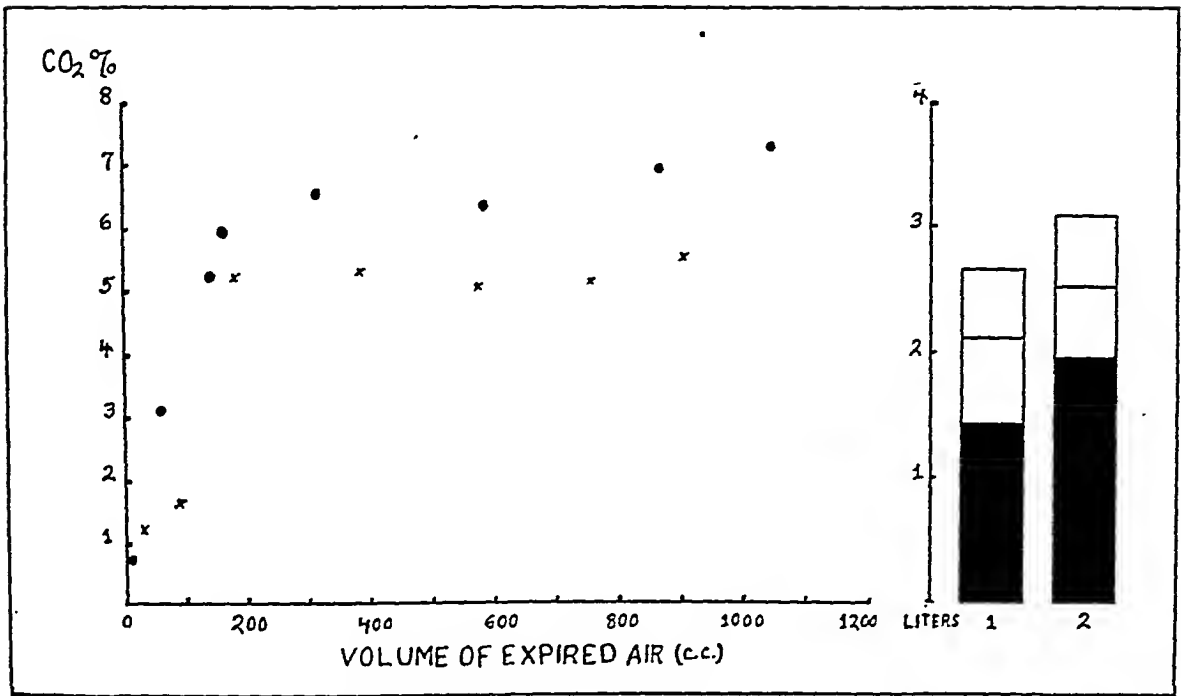


FIG. 4. CO<sub>2</sub> CONTENT OF VARIOUS SAMPLES TAKEN AT KNOWN VOLUMES IN A SINGLE EXPIRATION.

Black dots are samples taken in a case of pulmonary fibrosis, and crosses in a normal male subject. Column 1 represents the residual volume (black area) and the volume of the expiration (white area). The line dividing the white area is the resting respiratory level; therefore the space above this line is the tidal volume and the space below is the reserve air. Column 2 represents similar measurements in the case of pulmonary fibrosis.

beading of the trunks due to chronic proliferative changes in associated lymphoid tissues; 4—enlargement of the mediastinal lymph nodes due to proliferation of the local connective tissues, and 5—late chronic proliferation and nodule formation in the finer connective tissues of the pulmonary parenchyma. In the evolution of these pathological changes one may visualize a gradual encroachment of the fibrosis upon the functional alveolar air space, resulting in a progressive decrease in the total and vital capacities. It is somewhat more difficult to visualize the mechanism by which the residual air is increased. It is possible that as some alveoli are obliterated by fibrosis others may expand to fill the space by a compensatory emphysema. Then too, the increase in rigidity of the structure of the lung may resist the normal deflating power of its elastic tissue. Christie (11) has recently indicated that the loss of elastic properties of the alveolar wall is the factor responsible for the increase in the residual air observed in pulmonary emphysema. Binger

(12) and Lundsgaard (13) explained a similar, but more moderate, increase in residual air occurring in cases of cardiac decompensation by a decrease in the elasticity of the alveolar walls. Hurtado, Kaltreider and McCann (14) observed an increase in residual air of normal men at low barometric pressure. Under the same conditions they observed intense congestion of the pulmonary capillaries of guinea pigs and suggested that this was the cause of decreased elasticity of the alveoli and resultant increase in residual air. These observations suggest that the increase in residual air in cases of pulmonary fibrosis may be explained on the basis of alterations in the elastic properties of the alveolar walls, but there is no conclusive evidence to support this hypothesis.

The intrapleural pressure was measured in three cases of our series and the results are presented in Table V. In two of these cases there was a history of asthmatic attacks and the values for pulmonary capacity were typical of emphysema. The intrapleural pressure of these subjects



TABLE V

*Intrapleural pressure in three cases of pulmonary fibrosis*

	Case 25*	Case 27*	Case 32†
	cm. H <sub>2</sub> O	cm. H <sub>2</sub> O	cm. H <sub>2</sub> O
Quiet breathing...	-3 to +1	+0.8 to +2.5	-3 to -5
Forced inspiration	-15	-6	-3.6
Forced expiration	+ 3	+3.8	-1 to -0.2

\* Cases 25 and 27 had a history of asthmatic attacks; pulmonary capacity determinations revealed marked emphysema.

† Cases 32 showed marked nodular lesions in the roentgenographic film. Pulmonary capacity revealed a marked decrease in the vital capacity and a moderate increase in the residual air.

was positive. In the remaining case, in which the roentgenographic study of the lungs revealed very extensive nodular lesions, there was a diminished fluctuation of the pressure on forced breathing. One of the important signs of decreased elasticity, according to Christie (11), is a decrease in the reserve volume when determined after a full inspiration, as compared with the same capacity measured after an ordinary tidal inspiration. We have found this sign in only 23 (43.4 per cent) of the 53 cases in which we sought to demonstrate it. When a decrease in reserve air was noted it varied from 0.10 liter to a total disappearance of the reserve air, but there was no correlation between the degree of change and the other values in the pulmonary capacity. These observations indicate that changes in elasticity of the lungs play a part in the abnormal respiratory mechanism of pulmonary fibrosis.

Although the relative importance of the anatomical and functional factors in the pathological physiology of pulmonary fibrosis cannot be settled at the present time, it is fair to assume that both contribute to the abnormalities in the different pulmonary capacities, the determination of which may give us a quantitative index of the limits of adaptation to increased ventilatory demands. The importance of the measurement of the vital capacity has been demonstrated by Peabody (15) and his coworkers. In pathological conditions in which the residual air is increased one would expect to find conditions unfavorable to efficient alveolar ventilation. In a previous communication (6) we have indicated the close relationship, be-

tween the ratio of residual air to total capacity and the degree of respiratory disability in cases of pulmonary emphysema; the same correlation exists in pulmonary fibrosis (Figure 5, Table VI). It is difficult to evaluate the changes in

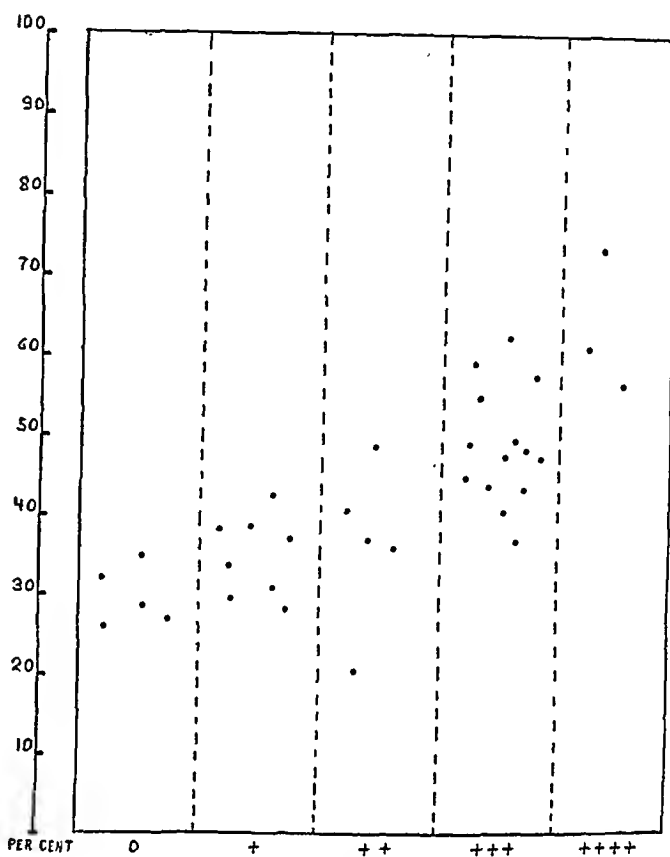


FIG. 5. CORRELATION BETWEEN THE DEGREE OF DYS-PNEA AND THE RATIO  $\frac{\text{RESIDUAL AIR}}{\text{TOTAL CAPACITY}} \times 100$  IN 35 CASES OF PULMONARY FIBROSIS.

0 = no dyspnea; + = dyspnea on severe physical exertion; ++ = on moderate exertion; +++ = on slight physical activity; and +++++ = dyspnea at rest.

total pulmonary capacity in relation to disability. However, from our experience with more than two hundred observations in normal subjects and in patients with chronic pulmonary disease it appears that a decrease of more than 15 per cent in the observed vital capacity is significant of abnormality; that moderate disability exists when it is 70 per cent of normal; and severe disability if it is below this limit. The ratio of residual air to total capacity, possibly abnormal when greater than 30 per cent, is definitely beyond the limits of normal if over 35 per cent, and is only moderately abnormal if not higher than 45 per cent. Beyond this limit the ratio is always associated

TABLE VI  
*Relation of dyspnea to pulmonary capacity*

Degree of dyspnea*	Number of cases	Percentage of normal vital capacity	Percentage of normal mid capacity	Percentage of normal residual air	Ratio Mid capacity Total capacity $\times 100$	Ratio Residual air Total capacity $\times 100$
		per cent	per cent	per cent		
0	5	-23.1	-14.6	+ 7.3	39.1	29.6
+	8	-31.9	- 0.1	+ 31.8	46.2	35.6
++	5	-36.3	+ 6.7	+ 32.6	52.4	36.4
+++	14	-50.9	+18.8	+ 66.9	60.0	49.1
++++	3	-67.9	+48.6	+119.2	76.1	63.6

\* 0 = no dyspnea; + = dyspnea on severe physical activity; ++ = on moderate exertion; +++ = on slight exertion; and ++++ = dyspnea at rest.

with a severe degree of respiratory disability and nearly always accompanied by a diminution in the saturation of the arterial blood with oxygen. Up to a certain point decrease in the vital capacity, if it is not accompanied by increase in the residual air, is compatible with a high degree of respiratory efficiency. In such a case the ratio, residual to total capacity, is not abnormally high.

It is interesting to compare the observations in pulmonary fibrosis and in pulmonary emphysema. These two conditions, apart from tuberculosis, represent the main types of chronic pulmonary disease in which respiratory disability is an important finding. Such a comparison is made in Table VII, in which we have included for comparative purposes the corresponding measurements

made in normal male subjects. In patients with emphysema the chest is larger and the diaphragm is held at a lower level than in those with pulmonary fibrosis, but in both conditions the chest tends to assume a rounded shape. The ability to expand the chest is slightly less in emphysema than in fibrosis. These two conditions seem to be chiefly differentiated by changes in the total pulmonary capacities. In emphysema the total capacity is almost normal due to the great increase in the residual air which compensates for the decrease in the vital capacity. The ratio of residual volume to total capacity is higher in this disease since two factors contribute to its alteration: the decrease in the vital capacity and the increase in the residual air. On the other hand, in pul-

TABLE VII  
*Comparison of the mean values for chest size and expansion and pulmonary capacity in normal male subjects (50 cases) pulmonary emphysema (24 cases) and pulmonary fibrosis (58 cases)*

	Normal	Emphysema	Fibrosis		Normal	Emphysema	Fibrosis
<i>Body measurements</i>				<i>Pulmonary capacity</i>			
Body height, cm.	176.2 $\pm$ 0.49	165.9 $\pm$ 1.39	163.5 $\pm$ 0.75	<i>Absolute values</i>			
Body weight, kgm.	72.5 $\pm$ 1.07	61.6 $\pm$ 1.82	65.6 $\pm$ 1.07	Total capacity, liters	6.13 $\pm$ 0.05	5.73 $\pm$ 0.14	4.61 $\pm$ 0.03
Body surface area, cm <sup>2</sup> .	187.8 $\pm$ 1.19	167.4 $\pm$ 2.88	172.2 $\pm$ 1.55	Vital capacity, liters	4.78 $\pm$ 0.05	2.88 $\pm$ 0.13	2.85 $\pm$ 0.06
<i>Size and shape of chest</i>				Mid capacity, liters	2.34 $\pm$ 0.03	3.68 $\pm$ 0.14	2.34 $\pm$ 0.03
Radiological chest volume, inspiration, liters	14.83 $\pm$ 0.17	14.77 $\pm$ 0.76	13.85 $\pm$ 0.22	Residual air, liters	1.37 $\pm$ 0.04	2.84 $\pm$ 0.14	1.71 $\pm$ 0.05
Anteroposterior expansion, cm.	6.1 $\pm$ 7.28	6.30 $\pm$ 12.52	6.33 $\pm$ 7.72	Complementary air, liters	3.79 $\pm$ 0.03	2.04 $\pm$ 0.08	2.26 $\pm$ 0.06
Lateral expansion, cm.	3.6 $\pm$ 0.20	24.2 $\pm$ 0.38	21.8 $\pm$ 0.27	Reserve air, liters	0.65 $\pm$ 0.02	0.54 $\pm$ 0.03	0.63 $\pm$ 0.03
Elevation of left diaphragm, inspiration, cm.	25.6 $\pm$ 0.23	25.1 $\pm$ 0.36	23.8 $\pm$ 0.23	<i>Relative values</i>			
Width of chest, inspiration, cm.	30.3 $\pm$ 0.16	25.3 $\pm$ 0.44	29.4 $\pm$ 0.22	Vital/Total capacity $\times 100$	78.0 $\pm$ 0.41	50.4 $\pm$ 1.47	63.0 $\pm$ 0.61
Depth of chest, inspiration, cm.	21.7 $\pm$ 0.18	23.1 $\pm$ 0.38	23.4 $\pm$ 0.22	Mid/Total capacity $\times 100$	37.9 $\pm$ 0.75	64.0 $\pm$ 1.52	50.9 $\pm$ 1.02
Chest index	68.5 $\pm$ 0.55	79.3 $\pm$ 1.47	77.1 $\pm$ 1.10	Residual/Total capacity $\times 100$	22.0 $\pm$ 0.41	49.5 $\pm$ 1.22	37.2 $\pm$ 0.63
<i>Chest expansion</i>				Complementary/Total capacity $\times 100$	61.9 $\pm$ 0.51	35.5 $\pm$ 1.47	49.3 $\pm$ 1.01
Anteroposterior expansion, cm.	3.9 $\pm$ 0.05	2.1 $\pm$ 0.12	1.9 $\pm$ 0.07	Reserve/Total capacity $\times 100$	16.2 $\pm$ 0.37	14.6 $\pm$ 0.25	13.3 $\pm$ 0.64
Lateral expansion, cm.	3.2 $\pm$ 0.05	2.4 $\pm$ 0.13	2.2 $\pm$ 0.05	Complementary/Vital capacity $\times 100$	72.4 $\pm$ 0.59	70.8 $\pm$ 1.37	78.2 $\pm$ 0.67
Elevation right diaphragm, cm.	6.3 $\pm$ 0.11	3.6 $\pm$ 0.22	4.2 $\pm$ 0.17	Reserve/Vital capacity $\times 100$	20.6 $\pm$ 0.53	29.2 $\pm$ 1.52	21.8 $\pm$ 0.69
Elevation left diaphragm, cm.	6.4 $\pm$ 0.12	4.4 $\pm$ 0.21	4.4 $\pm$ 0.17				
Rib rotation, degrees	21.4 $\pm$ 0.51	15.0 $\pm$ 0.79	14.4 $\pm$ 0.43				
Ratio Area at maximum expiration Area at maximum inspiration $\times 100$	62.2 $\pm$ 0.42	73.4 $\pm$ 1.20	73.0 $\pm$ 0.87				

The average ages of the different groups were as follows: Normal subjects, 23 years; pulmonary emphysema, 40 years; and pulmonary fibrosis, 47 years.

monary fibrosis, the total capacity is diminished. This is due to a marked decrease in the vital capacity while the residual air is only moderately increased; consequently the ratio  $\frac{\text{residual air}}{\text{total capacity}} \times 100$  is not as high as in emphysema. It is also interesting to notice that in emphysema the reduction in vital capacity occurs chiefly at the expense of the complementary air, while in fibrosis complementary and reserve air are about equally reduced. It has been our experience that the respiratory disability is also of greater severity in emphysema as compared with pulmonary fibrosis. Cases of pulmonary fibrosis which have developed emphysema are, as a rule, severely disabled.

#### SUMMARY AND CONCLUSIONS

Determinations of total pulmonary capacity and its subdivisions and measurements of the expansion of the chest have been made in 58 cases of pulmonary fibrosis, all but 4 of which gave a history of employment in dusty trades. Additional observations included a complete clinical examination, roentgenographic and electrocardiographic studies. The observations have been correlated with the degree of the anatomical changes in the lungs revealed by roentgenographs, with the probable etiological factor, and with the degree of respiratory disability. A comparison has also been made with previous observations from cases of pulmonary emphysema. These investigations lead to the following conclusions:

1. Definite alterations in the pulmonary capacity occur in cases of pulmonary fibrosis, consisting chiefly in moderately reduced total capacity, a more marked decrease in the vital capacity and a moderate or marked increase in the residual air.

2. The above changes are usually, but not always, proportional to the nature and extent of the pathological changes in the roentgenographs of the lungs. Frequent exceptions to this correlation indicate the necessity for studying and interpreting each case individually.

3. The changes in the pulmonary capacity are found to be correlated with the degree of clinical respiratory disability. The estimation of the vital capacity and of the ratio  $\frac{\text{residual air}}{\text{total capacity}} \times 100$  are

important indices of respiratory efficiency; the lower the former and the higher the latter, the greater the disability.

4. No significant alteration in the volume of the respiratory dead space was found in 12 cases of pulmonary fibrosis.

5. There is a reduction in the ability to expand the chest in pulmonary fibrosis, usually, but not always, proportional to the degree of the anatomical changes revealed in roentgenographs of the chest.

6. Comparison of the pulmonary capacities in uncomplicated cases of pulmonary fibrosis and in emphysema reveal significant differences. In uncomplicated fibrosis the total capacity and vital capacity are reduced, and the residual air is moderately increased. In emphysema the total capacity is normal, due to the fact that the increase in residual air compensates for the decrease in vital capacity.

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# STUDIES OF TOTAL PULMONARY CAPACITY AND ITS SUBDIVISIONS

## IX. RELATIONSHIP TO THE OXYGEN SATURATION AND CARBON DIOXIDE CONTENT OF THE ARTERIAL BLOOD<sup>1</sup>

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An index of the efficiency of the exchange of carbon dioxide and oxygen between the circulating blood and the alveolar air may be derived from the analysis of the arterial blood. However, it must be born in mind that changes in the gaseous content of the blood leaving the lungs may be brought about by a multiplicity of respiratory and circulatory factors, so that the proper interpretation of the results presents considerable difficulties. Although numerous analyses of arterial blood have been made in various pulmonary conditions, a review of the literature reveals very few attempts to correlate these studies with simultaneous studies of the external respiration. This is particularly true in the case of chronic pulmonary diseases, the fibroses and emphysema of the lungs. We have presented in previous communications (1) (2), evidence showing that the changes observed in the total pulmonary capacity and its subdivisions in cases of pulmonary emphysema and fibrosis are closely related to the anatomical alterations and tend to be correlated with the degree of clinical respiratory disability. The purpose of this communication is to correlate the alterations in the gaseous content of the arterial blood with the pulmonary capacities in 37 cases of pulmonary fibrosis and in 24 cases of pulmonary emphysema.

### REVIEW OF THE LITERATURE

The feasibility of obtaining arterial blood by puncture was first demonstrated by Hürter (3) in 1912, and several years later Stadie (4) in 1919, introduced the technique into this country.

<sup>1</sup> The expenses of this investigation were defrayed from a fund contributed by the Pfaudler Company, The Corning Glass Company, The Eastman Kodak Company, The American Grinding Wheel Manufacturing Association, The American Laundry Machinery Company, The Gleason Works and The Symington Company.

*Normal values.* A summary of the investigations made on the carbon dioxide content and oxygen saturation of the arterial blood of normal individuals is presented in Table I. A total of 37 observations on the oxygen saturation of healthy adult male subjects have been collected from the works of Harrop (5) in 1919, Meakins (6) in 1920, Himwich and Barr (7) and Barcroft and others (8) in 1923 and Hurtado; Kaltreider and McCann (9) recently. With the exception of three cases, two reported by Himwich and Barr and one by Meakins the arterial oxygen saturation has been found to be always above 94 per cent, so that this may be adopted as the lowest normal value. In Meakin's observation the subject was in poor physical condition at the time of the investigation.

There is less agreement in regard to the normal carbon dioxide content of the arterial (whole) blood than in the case of oxygen content. We have been able to collect 35 observations made on adult male subjects reported by Harrop (5) in 1919, Meakins (6) in 1920, Barr, Himwich and Green (10) in 1923, Burwell and Robinson (11) in 1924 and Hurtado, Kaltreider and McCann (9) in a recent publication. The results varied between 40.8 and 54.69 volumes per cent, and in consequence it appears difficult to establish the limits of normal variation. It is possible that technical errors may account for some of the abnormally high and low levels reported and it must also be remembered that the amount of this gas in the arterial blood is susceptible to variations depending on abnormalities in the breathing at the time when the blood is drawn. The limits of normal variation may be placed, somewhat arbitrarily, at 42.0 and 52.0 volumes per cent.

*Observations in pulmonary diseases.* Numerous analyses of arterial blood have been made

TABLE I

*Normal values for CO<sub>2</sub> content and O<sub>2</sub> saturation of the arterial blood (collected from the literature)*

Investigators	Number of cases	CO <sub>2</sub> content		O <sub>2</sub> saturation	
		Average	Variations	Average	Variations
		<i>volumes per cent</i>	<i>volumes per cent</i>	<i>per cent</i>	<i>per cent</i>
Harrop (1919).....	10	49.69	44.58 to 54.69	96.4	94.3 to 100
	15				
Meakins (1920).....	7	52.7	52.1 to 54.2	95.3	93.8 to 96.3
	11				
Barr, Himwich, Green (1923).....	8	47.85	40.8 to 50.9		
Himwich, Barr (1923).....	5			94.4	93.2 to 96.1
Barcroft and others (1923).....	4			95.5	95 to 97
Burwell and Robinson (1924).....	8	46.68	44.75 to 50.30		
Authors (1934).....	2	46.82	43.71 to 49.97	97.5	95.3 to 99.7

in cases of pneumonia. A decrease in the oxygen saturation has been found to occur in most cases at some stage of the disease, and evidence of this change has been presented by Stadie (4) (12) in 1919 and 1922, Barach and Woodell (13) and Meakins (14) in 1921, Hastings, Neill, Morgan and Binger (15) in 1924, Binger, Hastings and Sendroy (16) in 1927, Binger and Davis (17), Davis (18) and Binger (19) in 1928. That no significant change occurs in the carbon dioxide content of the arterial blood of patients with pneumonia has been reported by some of these investigators (15) (16). That a decrease in the oxygen saturation of the arterial blood is a frequent finding in cases of pulmonary emphysema has been demonstrated by several observers: Meakins (14) in 1921, Campbell, Hunt and Poulton (20) in 1923, Himwich and Loebel (21) in 1928, Kountz, Alexander and Dowell (22) in 1929 and very recently by Christie (23). An increase in the carbon dioxide content of the arterial blood in this disease has been found by Scott (24) and Meakins and Davies (25).

Few investigations of the gas content of the arterial blood have been made in pulmonary tuberculosis. Dautrebande (25) in 1925 observed no alteration in incipient cases, while in those patients with advanced and extensive lesions the oxygen saturation was reduced and the carbon dioxide content increased. Identical results were obtained by Pomplun (26) in 1928, while Hilton

(27) failed to find any significant abnormality of these values in nine cases.

Still fewer observations have been made in cases of pulmonary fibrosis. Of the three cases studied by Cossio and Berconsky (28) in 1932 only one appears to be uncomplicated by emphysema, and in this case the saturation of the arterial blood was reduced to 75.2 per cent. In five cases of pneumokoniosis studied in the copper mines of the Peruvian Andes, at 15,000 feet, one of us (A. H.) observed an average arterial saturation of 77.8 per cent, in contrast with the corresponding normal value of 83.4 per cent for such an altitude, while the carbon dioxide content was not significantly altered, taking into consideration the normal value found at that altitude (unpublished observations).

It has been assumed (29) (30), that edema of the alveolar walls causes marked alterations in the diffusion of the respiratory gases, and this assumption seems to be corroborated by experimental work on animals (31) (32).

#### MATERIAL AND METHODS

Analyses of the carbon dioxide content and of the oxygen saturation of the arterial blood have been made in 37 cases of pulmonary fibrosis and in 24 cases of pulmonary emphysema. All of the subjects were adult males. A complete presentation of the clinical and roentgenographic studies, and of the different measurements in chest ex-

TABLE II  
Carbon dioxide content and oxygen saturation of arterial blood in cases of pulmonary emphysema

Case number	Arterial Blood				Pulmonary Capacity							
	CO <sub>2</sub> content	O <sub>2</sub> content	O <sub>2</sub> capacity	O <sub>2</sub> saturation	Vital capacity		Mid capacity		Residual air		Ratio Residual air Total capacity	Ratio Mid capacity Total capacity
					Observed value	Difference from normal	Observed value	Difference from normal	Observed value	Difference from normal		
<i>volumes per cent</i>	<i>volumes per cent</i>	<i>volumes per cent</i>	<i>per cent</i>	<i>liters</i>	<i>per cent</i>	<i>liters</i>	<i>per cent</i>	<i>liters</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	
1	50.19	19.08	19.94	95.6	4.95	- 7.9	3.72	+41.9	2.64	+74.8	34.8	49.1
2	49.30	17.30	23.70	72.9	1.72	-60.9	5.36	+150.4	4.54	+267.7	72.5	85.6
3	48.81	19.16	21.63	88.6	2.34	-54.0	4.67	+88.3	3.87	+170.6	62.4	75.2
4	41.00	21.37	21.84	97.7	3.52	-26.5	3.09	+38.5	2.25	+66.6	39.0	53.5
5	43.01	20.60	21.40	96.4	3.76	-26.9	3.36	+36.8	2.33	+60.7	38.2	48.1
6	43.51	20.56	22.09	93.1	3.50	-30.2	2.67	+ 9.4	2.05	+45.4	36.9	48.1
7	51.32	17.88	19.94	89.7	2.78	-39.3	3.48	+55.1	2.50	+93.9	47.3	65.9
8	46.67	20.40	25.50	80.0	2.70	-52.3	6.56	+137.6	5.82	+266.6	68.3	77.0
9	47.06	20.46	22.57	90.6	3.18	-38.2	3.10	+23.5	2.28	+57.2	42.2	57.4
10	47.59	20.52	25.76	79.6	3.05	-54.2	4.67	+43.7	3.59	+90.9	54.1	70.4
11	49.00	18.60	19.90	93.4	1.94	-60.6	3.81	+58.7	2.83	+103.6	59.3	79.8
12	45.99	17.66	20.61	85.7	2.34	-55.4	3.51	+37.1	3.15	+112.8	57.3	63.9
13	43.76	20.91	23.91	87.4	3.06	-38.3	3.18	+31.4	2.66	+90.0	46.5	55.5
14	48.96	16.07	19.08	84.2	2.68	-46.0	4.30	+77.5	3.68	+162.8	57.8	67.6
15	40.73	22.80	23.93	95.3	3.54	-22.3	2.65	+19.3	1.63	+27.3	31.5	51.2
16*	55.33	17.52	23.58	74.3	0.86	-85.4	3.87	+34.8	3.61	+117.4	80.7	86.5
17*	48.19	17.37	21.16	82.1	1.20	-81.5	3.14	-0.9	2.96	+61.7	71.1	75.4
18†	55.59	15.69	19.26	81.5	2.09	-64.1	6.25	+120.0	5.77	+251.8	73.4	79.5
19†	44.04	14.34	15.58	92.0	1.68	-59.1	3.12	+56.0	2.80	+141.4	62.5	69.6
20†	45.71	20.01	21.73	92.0	3.45	-37.5	4.93	+83.2	4.23	+172.9	55.0	64.2
21†	48.66	19.91	21.62	92.1	2.08	-45.9	3.16	+68.9	2.86	+164.8	57.9	63.9
22†	42.34	17.69	19.65	89.9	2.92	-33.5	2.17	+ 1.4	1.71	+39.0	36.9	46.8
23†	45.11	16.34	18.18	89.9	2.12	-54.0	2.75	+22.8	1.91	+46.9	47.4	68.2
24†	58.16	17.57	20.31	86.5	1.66	-68.0	3.46	+36.7	2.58	+76.7	60.9	81.5

\* Diagnosis of emphysematous cysts also made.

† Diagnosis of pulmonary fibrosis also made.

pansion and pulmonary capacity of these patients has been made in previous publications (1) (2), and need no repetition here. Most of the cases of pulmonary fibrosis belonged to the group of pneumonokoniosis. The blood was obtained by puncture of the radial artery after the patients had had a preliminary rest of at least 15 minutes, and just prior to the determination of pulmonary capacity. Most of the patients were in the recumbent position. The usual precautions were observed to prevent exposure of the blood to the air. The analyses were carried out according to the method of Van Slyke and Neill (33) in their manometric apparatus, within two or three hours after the blood had been drawn.

#### OBSERVED VALUES OF THE OXYGEN SATURATION AND CARBON DIOXIDE CONTENT OF THE ARTERIAL BLOOD

The results of the determinations of the blood gases, together with the most important findings

in regard to the pulmonary capacities of all cases studied are presented in Tables II, and III, and summarized for both pulmonary fibrosis and emphysema in Table IV. A comparison of the findings in both diseases is made in Figure 1.

*Oxygen saturation.* Twenty-four cases of pulmonary emphysema varied between 72.9 and 97.7 per cent saturation of the arterial blood with oxygen with a mean value of 88.2 per cent, a value which is very definitely less than normal. In twenty cases (83.3 per cent) it was below the lower normal limit, 94 per cent, and in fourteen of these (58.8 per cent) the saturation was less than 90 per cent. The mean value for the oxygen combining capacities was well within the normal limits in this group of patients with emphysema but the oxygen content was significantly decreased.

In the thirty-seven cases of pulmonary fibrosis the mean value for the oxygen saturation of the arterial blood was 92.0 per cent. It varied between 80.6 and 98.5 per cent, and in twenty-six

TABLE III

*Carbon dioxide content and oxygen saturation of arterial blood in cases of pulmonary fibrosis*

Case number	Arterial Blood				Pulmonary Capacity							
	CO <sub>2</sub> content	O <sub>2</sub> content	O <sub>2</sub> capacity	O <sub>2</sub> saturation	Vital capacity		Mid capacity		Residual air		Ratio Residual air Total capacity	Ratio Mid capacity Total capacity
					Observed value	Difference from normal	Observed value	Difference from normal	Observed value	Difference from normal		
	<i>volumes per cent</i>	<i>volumes per cent</i>	<i>volumes per cent</i>	<i>per cent</i>	<i>liters</i>	<i>per cent</i>	<i>liters</i>	<i>per cent</i>	<i>liters</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
1	44.25	21.14	22.08	95.7	3.02	-36.0	2.53	+10.0	1.79	+34.6	37.2	52.5
2	46.20	18.88	23.25	81.2	2.32	-41.8	1.85	- 5.1	1.31	+15.9	36.0	50.9
3	39.62	19.30	20.32	95.4	2.10	-52.0	2.06	- 3.7	1.72	+38.7	45.0	53.9
4	42.70	19.39	21.08	92.0	1.54	-55.7	1.55	- 8.3	1.21	+23.5	44.0	56.3
5	44.95	20.80	22.46	92.7	3.00	-29.2	2.68	+30.1	1.94	+63.0	39.2	54.2
6	46.62	17.93	20.69	86.6	2.18	-62.9	2.87	0	2.13	+26.0	49.4	66.6
7	43.85	20.23	22.34	91.0	3.90	-25.4	4.80	+88.2	3.16	+115.0	44.8	67.9
8	42.09	20.88	22.76	91.7	2.78	-30.0	1.33	-31.1	1.11	- 0.8	28.5	34.1
9	42.00	21.60	22.57	95.7	2.68	-41.3	2.10	- 5.8	1.68	+30.3	38.5	48.1
10	43.87	19.26	20.77	92.2	2.22	-41.7	2.65	+48.6	2.11	+97.2	48.7	61.2
11	47.39	19.99	22.90	87.3	2.24	-50.3	2.23	+ 1.4	1.75	+37.8	43.8	55.8
12	44.73	17.50	20.37	85.9	2.30	-42.3	2.03	+ 4.1	1.71	+51.3	42.6	50.6
13	43.34	21.35	22.54	94.7	2.84	-29.1	1.79	- 8.2	1.37	+21.2	32.6	42.5
14	47.63	18.17	19.87	91.4	3.36	-27.2	3.00	+33.3	2.48	+92.2	42.5	51.3
15	47.53	20.35	21.95	92.7	3.08	-39.9	3.03	+21.2	2.09	+44.1	40.4	58.6
16	55.21	21.18	26.27	80.6	1.44	-71.8	2.22	-10.8	1.86	+29.1	56.4	67.3
17	46.54	18.67	19.98	93.4	2.92	-30.4	2.08	+ 1.9	1.14	- 3.4	28.1	51.2
18	41.40	19.50	20.82	93.6	3.26	-34.5	2.94	+21.4	2.20	+57.1	40.3	53.8
19	46.15	17.04	18.70	91.1	2.88	-28.7	1.98	+ 0.5	1.14	0	28.3	49.2
20	39.35	19.79	21.16	93.5	3.49	-28.2	2.95	+24.4	2.35	+71.5	40.3	50.5
21	46.57	18.20	19.59	92.9	3.15	-33.4	2.44	+ 6.0	1.84	+38.3	36.9	48.9
22	45.11	22.45	24.86	90.3	1.56	-59.3	1.34	-28.3	1.14	+ 5.5	42.3	49.6
23*	41.75	21.20	22.57	93.9	3.26	-33.4	2.20	- 7.9	1.52	+10.1	31.8	45.9
24	48.37	18.42	20.04	91.9	2.20	-43.3	1.84	- 2.6	0.68	-37.6	23.6	63.8
25	47.80	18.70	19.78	94.5	3.52	-14.1	2.21	+10.5	1.57	+35.3	30.9	43.4
26	40.65	21.85	22.61	95.0	3.68	-20.6	1.66	-26.2	1.22	- 6.1	24.9	33.8
27	44.08	17.75	18.92	93.9	4.04	-13.7	2.45	+ 7.4	1.69	+28.0	29.5	42.8
28	43.08	20.88	22.76	91.7	3.18	-29.7	1.26	-42.7	0.94	-25.9	22.8	30.6
29	46.86	20.94	24.13	86.7	1.72	-67.6	2.54	- 1.9	2.38	+52.0	58.0	61.9
30	42.09	16.89	18.07	93.5	3.55	-26.0	2.15	- 8.1	1.65	+22.0	31.7	41.3
31	43.26	19.91	20.67	96.3	3.79	-14.6	2.04	- 5.5	1.32	+ 5.6	25.8	39.9
32	42.85	19.79	20.22	97.8	3.06	-26.8	1.55	-23.6	0.99	-15.4	24.4	38.2
33	45.66	19.92	20.70	96.2	3.08	-31.1	2.83	+29.8	2.27	+80.1	42.4	52.9
34	46.07	19.58	20.72	94.5	3.96	-28.3	3.55	+31.9	2.53	+63.2	38.9	54.7
35	44.37	18.75	20.15	93.0	3.14	-29.9	1.75	-19.7	1.13	-10.3	26.4	40.9
36	42.90	19.00	19.28	98.5	2.73	-32.2	1.57	-19.9	0.87	-23.0	23.9	43.2
37	51.83	16.39	17.49	93.7	2.26	-57.3	3.91	+51.4	3.07	+106.0	57.6	73.4

\* Diagnosis of bronchiectasis also made.

TABLE IV

*Summary of the values with respect to arterial blood and pulmonary capacity in cases of pulmonary fibrosis and emphysema*

	Mean	Pulmonary Fibrosis (37 cases)			Pulmonary Emphysema (24 cases)			
		Standard deviation	Coefficient of variation	Variations	Mean	Standard deviation	Coefficient of variation	Variations
<i>Arterial Blood</i>								
CO <sub>2</sub> content, volumes per cent.....	44.89±0.34	3.06	6.8	39.35-55.21	47.67±0.69	4.38	9.2	43.73-58.16
O <sub>2</sub> content, volumes per cent.....	19.50±0.16	1.44	7.4	16.37-22.45	18.75±0.27	1.96	10.4	14.34-22.89
O <sub>2</sub> capacity, volumes per cent.....	21.20±0.20	1.83	8.6	17.49-26.27	21.29±0.24	2.22	10.4	15.58-25.76
O <sub>2</sub> saturation, per cent.....	92.0 ±0.47	3.9	4.2	80.6-98.5	88.2 ±0.92	6.7	7.6	72.9-97.7
<i>Pulmonary Capacity</i>								
Vital capacity, liters.....	2.87±0.07	0.69	24.0	1.44- 4.04	2.63±0.13	0.92	34.9	0.56- 4.98
Mid capacity, liters.....	2.32±0.08	0.73	31.4	1.33- 4.50	3.79±0.14	1.02	26.9	2.17- 6.16
Residual air, liters.....	1.71±0.06	0.56	32.7	0.68- 3.16	3.17±0.15	1.09	34.9	1.63- 5.92
Ratio residual/Total capacity, per cent.....	37.1 ±1.07	9.7	26.1	22.8-58.0	53.7 ±1.52	13.2	24.6	31.5-81.7
Ratio Mid/Total capacity, per cent.....	50.7 ±1.08	9.8	19.3	30.6-73.4	66.0 ±1.72	12.5	18.9	46.8-80.5



cases (70.3 per cent) it was below the lower normal limit of 94 per cent. In contrast with the group having emphysema it was below 90 per cent in only six patients, 16.2 per cent. These results show that the saturation of the arterial blood with oxygen is affected less in cases of pulmonary fibrosis than in those with emphysema. The oxygen combining power was almost identical in both groups of cases but in the group with

between 40.73 and 58.16 volumes per cent. The values observed were scattered between the normal limits of variation, and in only three cases did the results indicate an abnormally high content.

In the patients with pulmonary fibrosis the mean value observed was 44.89 volumes per cent with variations between 39.35 and 55.21 volumes per cent. Referring to Figure 1, it will be noted that most of the results obtained in this condition

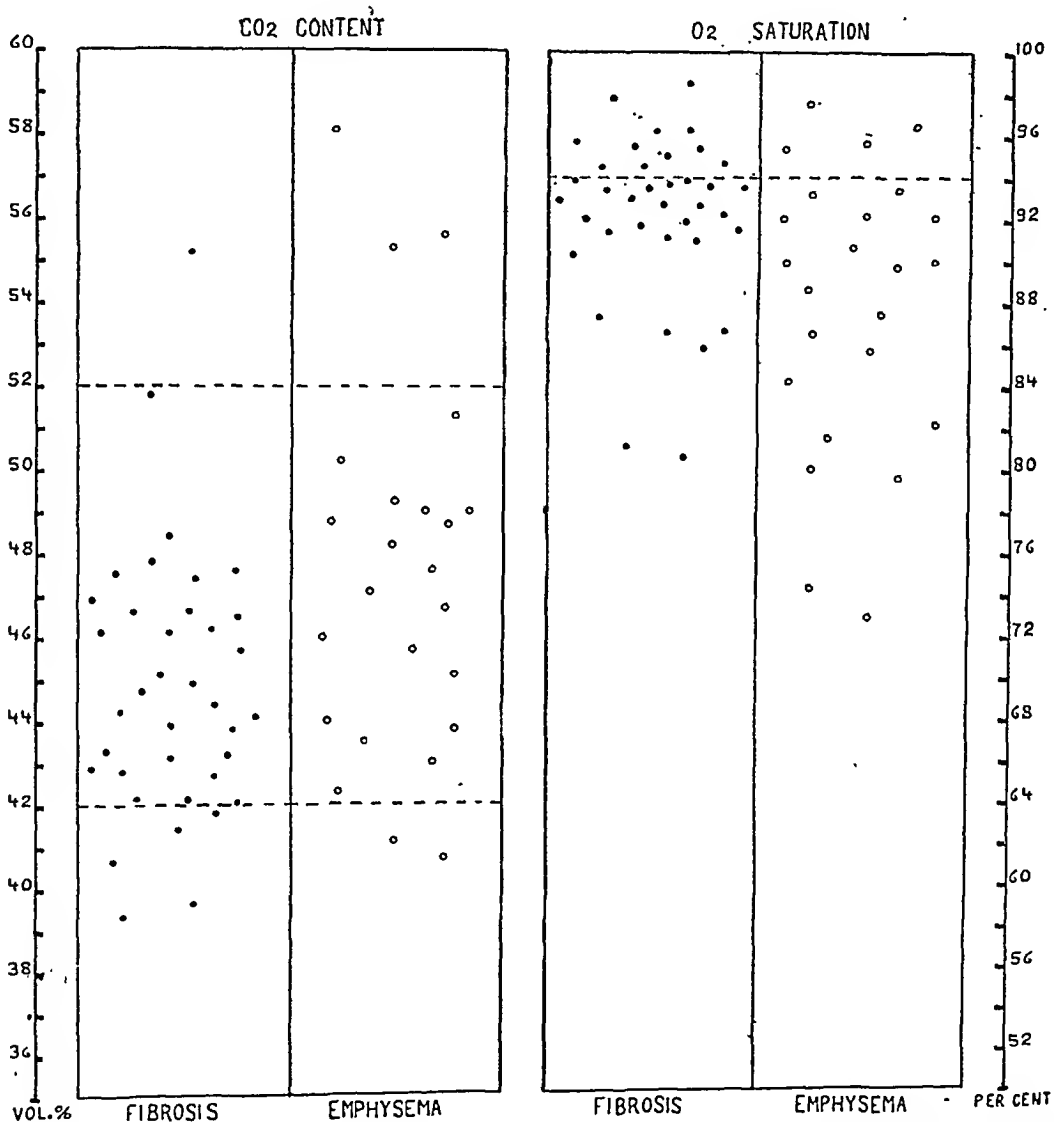


FIG. 1. CARBON DIOXIDE CONTENT AND OXYGEN SATURATION OF THE ARTERIAL BLOOD IN 24 CASES OF PULMONARY EMPHYSEMA (CIRCLES) AND 37 CASES OF PULMONARY FIBROSIS (DOTS).

fibrosis the average oxygen content of the arterial blood was higher.

*Carbon dioxide content.* The mean value for the carbon dioxide content of the arterial blood in the cases of pulmonary emphysema was 47.67 volumes per cent, with the extreme variations

were grouped near the lower normal limits of variation, and in one case only was the value abnormally high. These observations indicate that a higher carbon dioxide content is found in cases of pulmonary emphysema than is usual with those of fibrosis.

TABLE V

*O<sub>2</sub> saturation of the arterial blood and its relationship to the pulmonary capacity in cases of pulmonary fibrosis and emphysema*

O <sub>2</sub> saturation	Average Values							
	Vital capacity		Mid capacity		Residual air		Ratio	
	<i>per cent from normal</i>		<i>per cent from normal</i>		<i>per cent from normal</i>		$\frac{\text{Residual air}}{\text{Total capacity}} \times 100$	
	Fibrosis	Emphysema	Fibrosis	Emphysema	Fibrosis	Emphysema	Fibrosis	Emphysema
<i>per cent</i>								
94 to 100.....	−29.6	−20.9	+1.0	+34.1	+24.0	+ 57.3	33.1	35.9
90 to 94.....	−34.8	−45.2	+9.3	+49.9	+34.8	+114.2	36.4	52.3
85 to 90.....	−55.7	−48.9	+0.9	+38.9	+41.8	+ 90.0	48.4	51.2
80 to 85.....	−56.8	−60.9	−7.7	+83.5	+22.5	+185.7	46.2	67.6
75 to 80.....		−54.2		+43.7		+ 90.9		54.1
70 to 75.....		−73.1		+92.6		+192.5		76.6

*Correlation of the gaseous content of the arterial blood with the pulmonary capacity*

**Oxygen saturation.** The relationship of the oxygen saturation of the arterial blood to the various observations of the pulmonary capacity is summarized in Table V. We have been especially interested in the comparison of the degree of anoxemia with the ratio  $\frac{\text{Residual air}}{\text{Total capacity}} \times 100$ . From observations which we have made in cases

of pulmonary emphysema and fibrosis it appears that this ratio gives, as a rule, a fair index of the abnormality of the respiratory mechanism in these conditions and that it is well correlated with the degree of disability.

A comparison of the degree of oxygen saturation of the arterial blood with the value of the ratio in cases of pulmonary emphysema is presented in Figure 2. It reveals a definite tendency for the saturation to decrease as the ratio in-

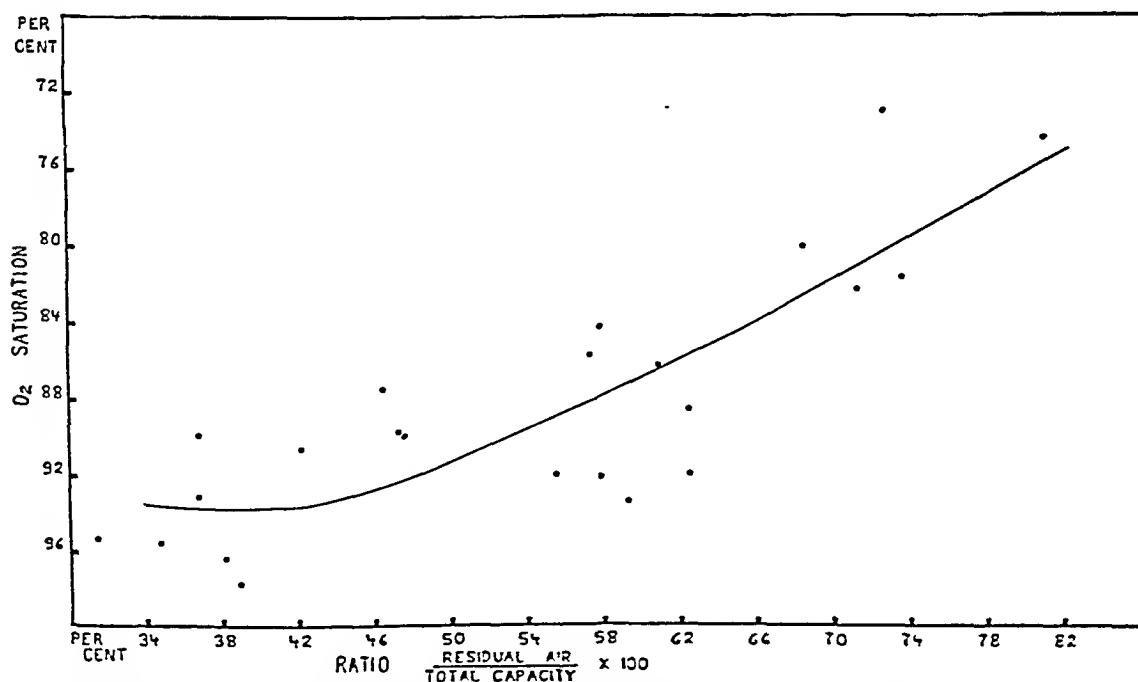


FIG. 2. CORRELATION OF THE OXYGEN SATURATION OF THE ARTERIAL BLOOD WITH THE RATIO OF RESIDUAL AIR TO TOTAL PULMONARY CAPACITY IN 24 CASES OF PULMONARY EMPHYSEMA.

Curve is a mathematically calculated logarithmic curve.

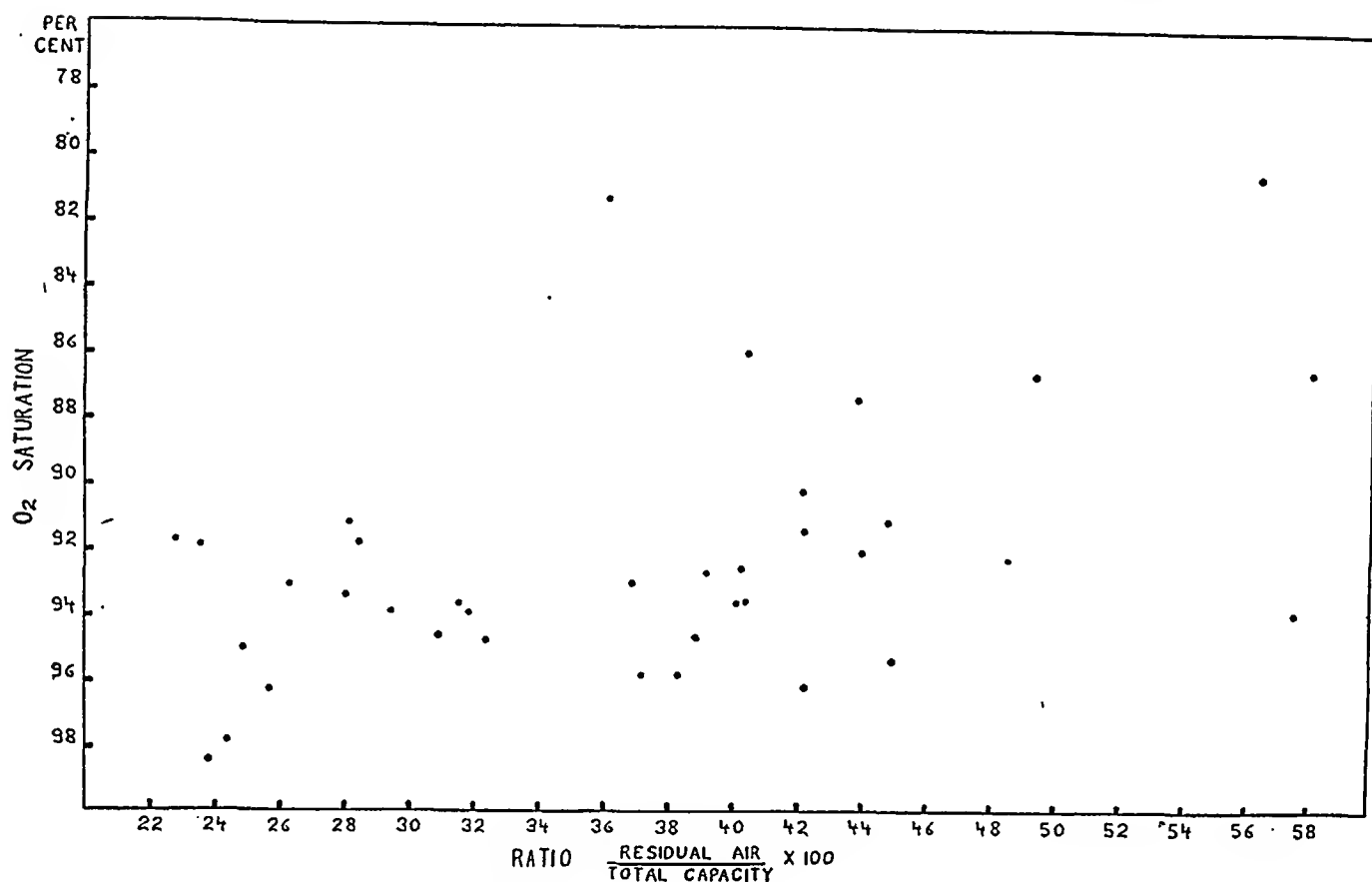


FIG. 3. COORDINATION OF THE OXYGEN SATURATION OF THE ARTERIAL BLOOD AND THE RATIO OF RESIDUAL AIR TO TOTAL PULMONARY CAPACITY IN 37 CASES OF PULMONARY FIBROSIS.

creases, although the relationship does not appear to be linear in character. Some degree of anoxemia was present in all instances in which the ratio exceeded 40 per cent. In all cases, but one, in which the ratio was greater than 60 per cent the degree of anoxemia was extreme. The relationship of these same factors in cases of pulmonary fibrosis is shown in Figure 3. Whenever the ratio  $\frac{\text{Residual air}}{\text{Total capacity}} \times 100$  exceeded 45 per

cent, all cases, but one, showed some degree of unsaturation, but below this limit there appeared to be no correlation between these two factors. The arterial blood may or may not be saturated with oxygen when the ratio was low, but the degree of anoxemia is very slight in those circumstances, being in no case below 91 per cent. In general the statement is true that when the residual air constitutes 45 per cent or more of the total capacity of the lungs it is usual to find some

TABLE VI

*CO<sub>2</sub> content of the arterial blood and its relationship to the pulmonary capacity in cases of pulmonary fibrosis and emphysema*

CO <sub>2</sub> content	Average Values							
	Vital capacity		Mid capacity		Residual air		Ratio	
	<i>per cent from normal</i>		<i>per cent from normal</i>		<i>per cent from normal</i>		$\frac{\text{Residual air}}{\text{Total capacity}} \times 100$	
	Fibrosis	Emphysema	Fibrosis	Emphysema	Fibrosis	Emphysema	Fibrosis	Emphysema
<i>volumes per cent</i>								
36 to 40.....	−40.1		+10.3		+55.1		42.6	
40 to 44.....	−31.5	−29.6	+ 2.1	+22.8	+22.2	+ 54.7	33.0	38.2
44 to 48.....	−37.0	−50.1	+ 7.4	+57.7	+36.3	+126.9	38.4	55.2
48 to 52.....	−50.3	−49.5	+24.4	+67.5	+34.2	+137.5	40.6	57.9
52 to 56.....	−71.8	−74.8	−10.8	+77.2	+29.1	+184.6	56.4	77.0
56 to 60.....		−68.0		+36.7		+ 76.7		60.9

degree of anoxemia of the arterial blood and that the degree of anoxemia tends to increase as the ratio increases beyond this limit.

*Carbon dioxide content.* In Table VI there is a comparative study of the carbon dioxide content

cases are grouped on the basis of the relative value of the residual air the average values observed of the oxygen saturation and carbon dioxide content of the arterial blood in each group show a striking relationship to the ratio. The

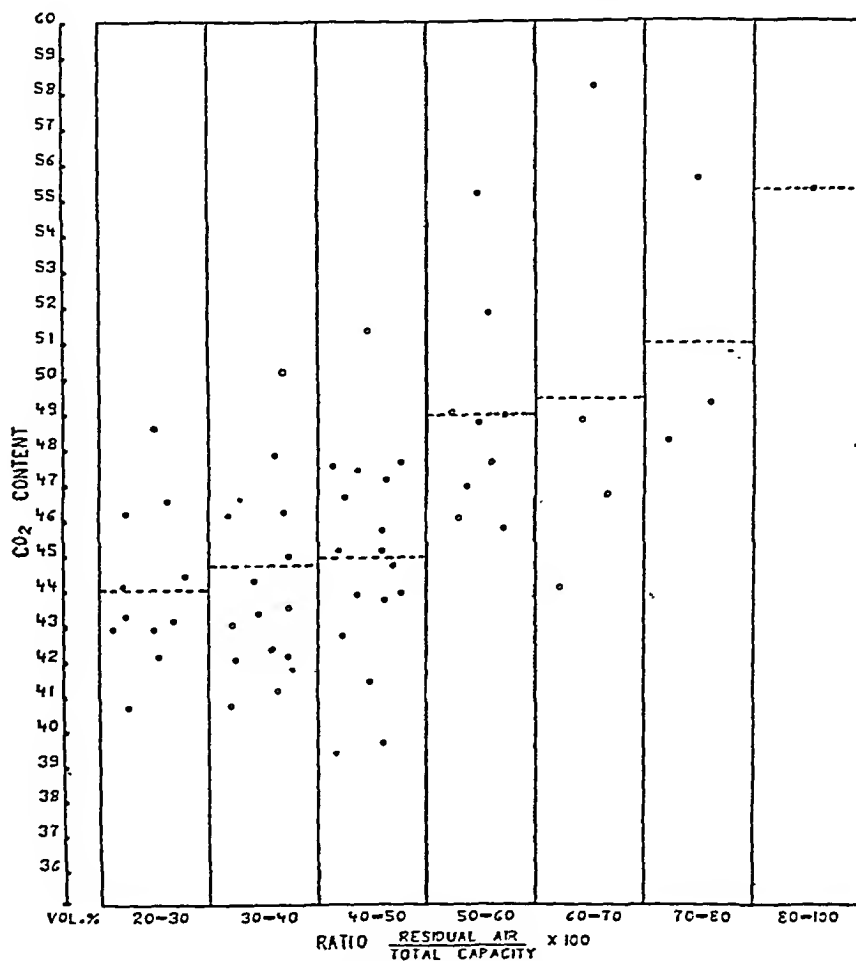


FIG. 4. CORRELATION OF THE CARBON DIOXIDE CONTENT OF THE ARTERIAL BLOOD TO THE RATIO OF RESIDUAL AIR TO TOTAL PULMONARY CAPACITY IN 24 CASES OF PULMONARY EMPHYSEMA (CIRCLES) AND 37 CASES OF PULMONARY FIBROSIS (DOTS).

Broken lines represent the average carbon dioxide content in each group.

of the arterial blood and the pulmonary capacity. The correlation between the ratio

$$\frac{\text{Residual air}}{\text{Total capacity}} \times 100$$

and the carbon dioxide content in all cases investigated, both of pulmonary emphysema and fibrosis, is presented in Figure 4, which shows a definite tendency for the carbon dioxide content to vary directly with the ratio. When all of the

critical value of the ratio at which the average values of the blood gases become definitely abnormal are between 40 and 50 per cent.

#### DISCUSSION

Although the elimination of carbon dioxide and the absorption of oxygen are mutually dependent processes in certain respects, they may be considered separately. Difficulty in the elimination

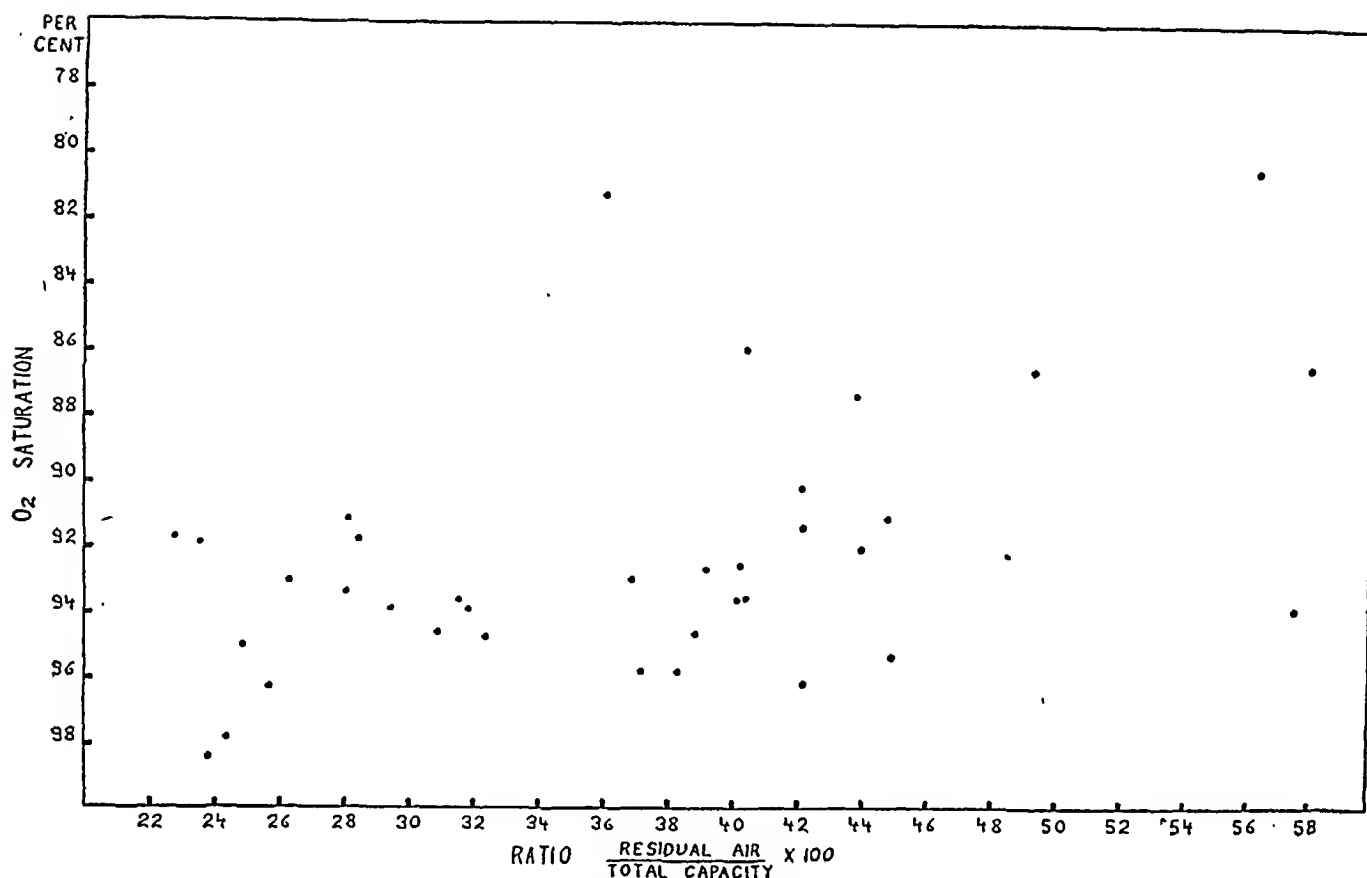


FIG. 3. COORDINATION OF THE OXYGEN SATURATION OF THE ARTERIAL BLOOD AND THE RATIO OF RESIDUAL AIR TO TOTAL PULMONARY CAPACITY IN 37 CASES OF PULMONARY FIBROSIS.

creases, although the relationship does not appear to be linear in character. Some degree of anoxemia was present in all instances in which the ratio exceeded 40 per cent. In all cases, but one, in which the ratio was greater than 60 per cent the degree of anoxemia was extreme. The relationship of these same factors in cases of pulmonary fibrosis is shown in Figure 3. Whenever the ratio  $\frac{\text{Residual air}}{\text{Total capacity}} \times 100$  exceeded 45 per

cent, all cases, but one, showed some degree of unsaturation, but below this limit there appeared to be no correlation between these two factors. The arterial blood may or may not be saturated with oxygen when the ratio was low, but the degree of anoxemia is very slight in those circumstances, being in no case below 91 per cent. In general the statement is true that when the residual air constitutes 45 per cent or more of the total capacity of the lungs it is usual to find some

TABLE VI

*CO<sub>2</sub> content of the arterial blood and its relationship to the pulmonary capacity in cases of pulmonary fibrosis and emphysema*

CO <sub>2</sub> content	Average Values							
	Vital capacity		Mid capacity		Residual air		Ratio Residual air Total capacity × 100	
	per cent from normal		per cent from normal		per cent from normal			
	Fibrosis	Emphysema	Fibrosis	Emphysema	Fibrosis	Emphysema	Fibrosis	Emphysema
volumes per cent								
36 to 40.....	—40.1		+10.3		+55.1		42.6	
40 to 44.....	—31.5	—29.6	+ 2.1	+22.8	+22.2	+ 54.7	33.0	38.2
44 to 48.....	—37.0	—50.1	+ 7.4	+57.7	+36.3	+126.9	38.4	55.2
48 to 52.....	—50.3	—49.5	+24.4	+67.5	+34.2	+137.5	40.6	57.9
52 to 56.....	—71.8	—74.8	—10.8	+77.2	+29.1	+184.6	56.4	77.0
56 to 60.....		—68.0		+36.7		+ 76.7		60.9

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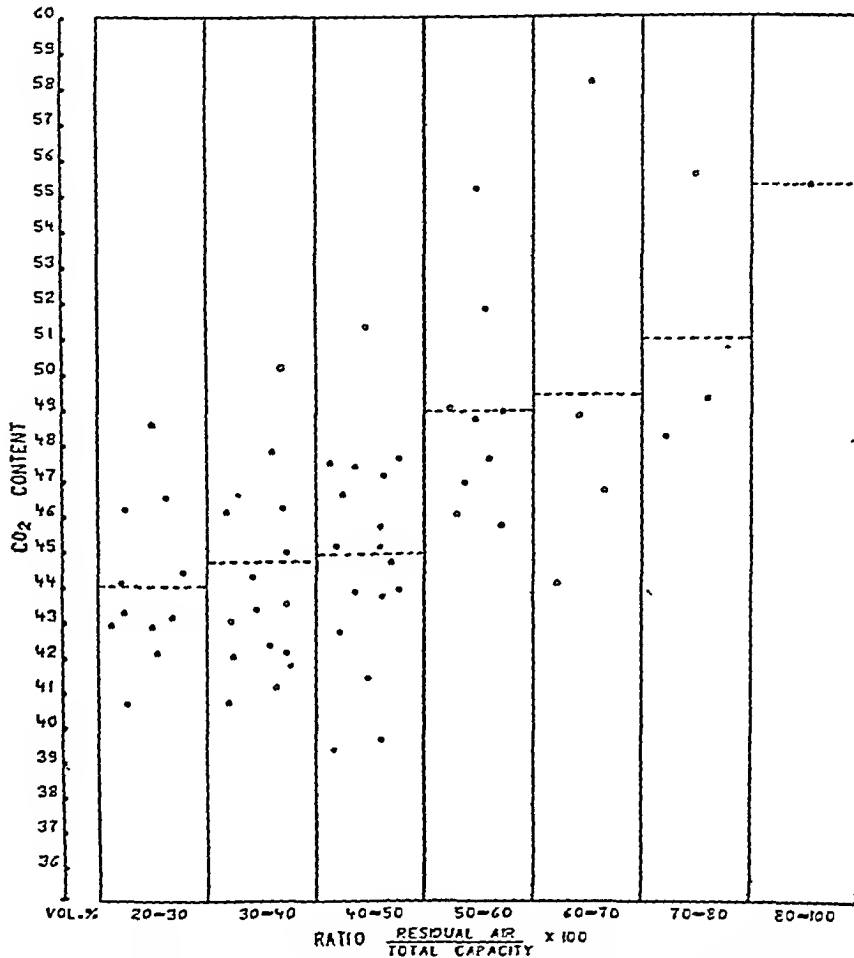


FIG. 4. CORRELATION OF THE CARBON DIOXIDE CONTENT OF THE ARTERIAL BLOOD TO THE RATIO OF RESIDUAL AIR TO TOTAL PULMONARY CAPACITY IN 24 CASES OF PULMONARY EMPHYSEMA (CIRCLES) AND 37 CASES OF PULMONARY FIBROSIS (DOTS).

Broken lines represent the average carbon dioxide content in each group.

of the arterial blood and the pulmonary capacity. The correlation between the ratio

$$\frac{\text{Residual air}}{\text{Total capacity}} \times 100$$

and the carbon dioxide content in all cases investigated, both of pulmonary emphysema and fibrosis, is presented in Figure 4, which shows a definite tendency for the carbon dioxide content to vary directly with the ratio. When all of the

critical value of the ratio at which the average values of the blood gases become definitely abnormal are between 40 and 50 per cent.

#### DISCUSSION

Although the elimination of carbon dioxide and the absorption of oxygen are mutually dependent processes in certain respects, they may be considered separately. Difficulty in the elimination

of carbon dioxide may be partly compensated by an increase in the alkaline reserve of the blood. If, however, some of the blood flowing through the lungs escapes complete aeration the mixed pulmonary blood in the left auricle will be incompletely saturated and arterial anoxemia will be inevitable no matter how much the ventilation and blood flow through normal portions of the lung may be increased.

The factors (apart from a low barometric pressure) which will operate in causing a diminution in the oxygen saturation of the arterial blood are numerous, but they may be ultimately considered from three general points of view: (1) deficient alveolar ventilation (lack of proper mixing, anatomical alterations preventing a free flow of air, shallow breathing); (2) reduced permeability for diffusion (in the alveolar and capillary walls or in the blood itself), and (3) the influence of the abnormalities just mentioned will be modified by the preservation or suppression of the circulation through the affected lung zones. It is evident from this brief consideration that anatomical as well as functional abnormalities of the lung parenchyma may play an etiological rôle in the production of anoxemia, and we may investigate first the extent to which these abnormalities are present in pulmonary emphysema.

Christie (23) has recently published a most complete study of the functional pathology of emphysema. Our own observations of the pulmonary capacity in this condition have been published (1). In both papers the factors entering into the deficient alveolar ventilation and resultant anoxemia of this condition have been reviewed, particularly with reference to the loss of elasticity, the increase in intrapleural and venous pressure, the dilatation of the alveoli, the increase in residual air and the decrease in vital capacity. In the present paper we have endeavored to bring out the relationship between the last two factors and the gas content of the arterial blood.

There is much to suggest that there are great inequalities in the efficiency of ventilation of the alveoli in different parts of the lungs with the result that the mixed pulmonary blood in the left auricle is incompletely saturated with oxygen. By making fractional analyses of a single expiration, Nielson and Sonne (34) have recently demonstrated that the alveolar air is not uniform

in composition, even in normal individuals, and suggest that it is quite likely that this lack of uniformity is more pronounced in pulmonary disease. Further studies of this type are being carried on in our laboratory with an improved apparatus. Meakins (6) observed a decrease in the saturation of the arterial blood following the induction of shallow breathing in normal individuals, and expressed the opinion that it is an important mechanism in the anoxemia observed in patients with pneumonia (35). A study of the respiratory tracings in our cases does not disclose the presence of shallow breathing, and it appears that at least during rest it is not a significant factor in the altered haemo-respiratory exchange.

Alterations in the alveolar permeability probably play no rôle in the imperfect oxygenation of the blood in cases of emphysema. Krogh (36) in 1914 found a normal permeability and diffusion constant in three cases of emphysema. Although the possibility of a disturbed permeability cannot be entirely dismissed our observations suggest that deficiency in alveolar ventilation is probably the most important factor.

The explanation of the anoxemia found in pulmonary fibrosis is more difficult. Although there is some evidence (2) to indicate that alterations in elasticity of the lungs probably exist in this condition, the question has not been definitely settled. It is interesting to observe that anoxemia is mainly found when the ratio of residual air to total capacity is over 45 per cent. In this condition, as in pulmonary emphysema, an insufficient alveolar ventilation is a factor which accounts for the anoxemia in part. However, the nature of the anatomical changes in pulmonary fibrosis indicated that in well advanced cases there are dense areas which in all probability receive little or no ventilation, and, if the blood is incompletely shunted from these regions anoxemia will result. We have been unable to find a definite correlation between the degree of anoxemia and the extent of the fibrotic lesions as seen in the roentgenographic film (see Table VII).

Although considerable thickening of the alveolar walls is frequently found at autopsy in cases of pulmonary fibrosis, suggesting that a decreased diffusion of oxygen ("pneumoniosis") may exist in this condition, there is no experimental or direct proof in favor of this assumption.

TABLE VII

*CO<sub>2</sub> content and O<sub>2</sub> saturation of the arterial blood in cases of pulmonary fibrosis arranged in groups according to the roentgenographic appearance.\**

Groups*	Number of cases	CO <sub>2</sub> content		O <sub>2</sub> saturation	
		Average	Variations	Average	Variations
		volumes per cent	volumes per cent	per cent	per cent
Group I	17	43.72	39.35-47.80	94.1	91.4-98.5
Group III	12	45.08	39.62-55.21	90.1	80.6-96.3
Group IV	3	44.13	42.00-46.54	93.8	92.2-95.7
Group V	3	48.91	47.39-51.83	91.2	87.3-93.7
Group VI	2	47.61	46.86-48.37	89.3	86.7-91.9

\* Group I. Cases which showed increased linear markings in the lung fields.

Group III. Patients included in this group showed nodular shadows.

Group IV. In this group the nodular shadows showed a tendency to agglomerate, giving a mottled appearance.

Group V. Patients in this group presented large dense shadows chiefly in the upper portions of the lung and in addition showed marked emphysema at the bases of the lungs.

Group VI. A fine and diffuse reticular fibrosis involving the whole of the lung fields was present in the cases included in this group.

It should not be assumed that the existence of anoxemia indicates a high degree of disability. To be sure it represents a partial failure of respiratory function, however it has been observed that the natives of high altitudes may live in a condition of constant and marked anoxemia and still exhibit a most surprising adaptation to physical and mental work (37) (38). It is quite possible that the anoxemia which follows the development of chronic anatomical alterations in the lungs leads to some unknown adaptative mechanism, which in this way differs in its significance from the one which results from an acute loss of respiratory function, such as that which occurs in pneumonia, or as a result of a sudden decrease in barometric pressure.

The carbon dioxide content of the arterial blood seems also to have some relationship to the degree of the alterations in pulmonary capacity. An abnormal increase in the content of CO<sub>2</sub> is chiefly observed in cases of pulmonary emphysema in which these alterations are especially

marked. This suggests that the same functional abnormalities which have been mentioned in connection with the development of anoxemia are partly responsible for the accumulation of carbon dioxide. However, one finds a severe anoxemia not uncommonly accompanied by a normal carbon dioxide content of the arterial blood. The high carbon dioxide content usually found in cases of pulmonary emphysema is accompanied by an increase of blood bicarbonates. Since the work of Scott (24) this has been interpreted as a mechanism by means of which the emphysematous patient is able to compensate, at least in part, the difficulties in the elimination of carbon dioxide during physical activity.

#### SUMMARY AND CONCLUSIONS

The oxygen saturation and the carbon dioxide content of the arterial blood have been studied in 24 cases of pulmonary emphysema and in 27 cases of pulmonary fibrosis. Most of the latter were cases of pneumokoniosis. The findings have been correlated with the degree of alteration in the pulmonary capacity simultaneously determined, and lead to the following conclusions:

1. Anoxemia is a very frequent finding in cases of pulmonary fibrosis and emphysema, but it appears to be much more pronounced in the latter disease.

2. The carbon dioxide content of the arterial blood has been found to be increased chiefly in cases of pulmonary emphysema. Most cases of fibrosis exhibit a rather low normal content of this gas.

3. The degree of unsaturation of the arterial blood with oxygen appears to be definitely correlated with abnormalities in pulmonary capacity, this being chiefly evident in cases of emphysema.

4. When the residual air constitutes 45 per cent or more of the total capacity a certain degree of anoxemia is almost invariably present, both in pulmonary emphysema and fibrosis.

5. Abnormally high values of carbon dioxide in the arterial blood are found in association with a marked increase of the ratio of residual air to total capacity.

6. Though many factors must be considered in connection with the anoxemia of pulmonary emphysema and of pulmonary fibrosis a deficient alveolar ventilation is probably the most important



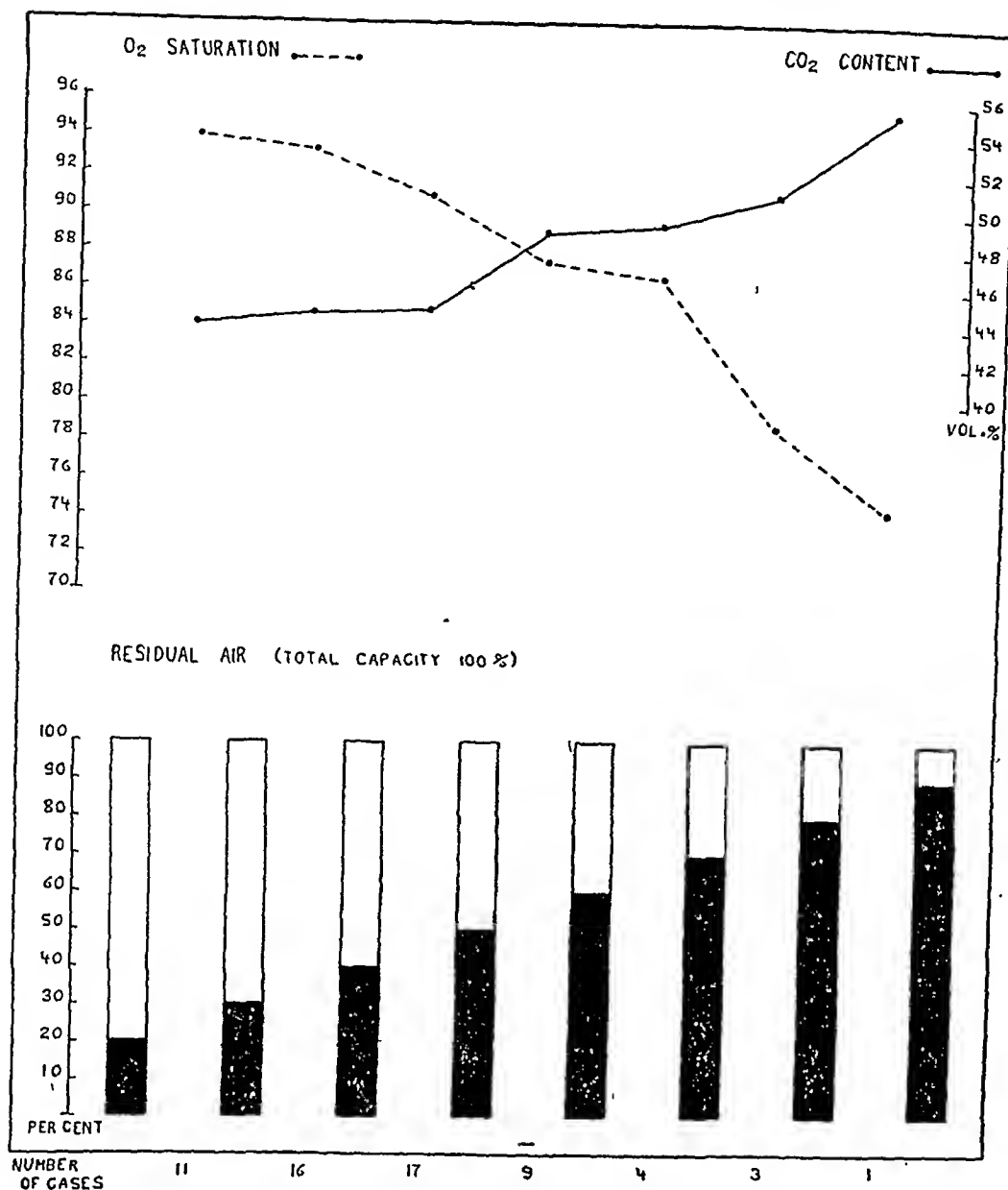


FIG. 5. AVERAGE OXYGEN SATURATION (BROKEN LINE) AND CARBON DIOXIDE CONTENT (SOLID LINE) OF THE ARTERIAL BLOOD OF 61 CASES OF PULMONARY EMPHYSEMA AND FIBROSIS GROUPED ACCORDING TO THE OBSERVED RATIO OF RESIDUAL AIR TO TOTAL CAPACITY.

factor, the best index of which is found in the ratio of residual air to total capacity.

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# CONCERNING THE NATURALLY OCCURRING PORPHYRINS

## I. THE ISOLATION OF COPROPORPHYRIN I FROM THE URINE IN A CASE OF CINCOPHEN CIRRHOSIS<sup>1</sup>

By CECIL JAMES WATSON

*(From the Department of Medicine, University of Minnesota Hospital, Minneapolis)*

(Received for publication September 10, 1934)

was painless. The stools were clay colored and the urine dark brown in color. There was marked ascites which recurred rapidly following each abdominal paracentesis. There was increasing edema of the legs. Hemorrhoids were noted. A definite hemorrhagic tendency was observed, characterized by petechiae and epistaxis. Neither liver nor spleen was palpable. The patient was apathetic, and during the last few days of life was difficult to arouse. Death occurred after a period of coma of about 36 hours duration. *Laboratory examinations:* The hemoglobin was 88 per cent (Sahli). The red blood cells numbered 4,500,000. Another hemoglobin determination one week before death was 96 per cent (Sahli). The leukocytes were 13,500, on another occasion 12,500; the neutrophils 84 per cent and the lymphocytes 16 per cent. The total leukocyte count one week before death had decreased to 7,800. The blood platelets were 200,000 per cu. mm. The coagulation time of blood obtained by ear lobe puncture was  $1\frac{1}{2}$  minutes; the bleeding time was 5 minutes. The capillary resistance test was strongly positive. The icterus index shortly after admission to the hospital was 140 units. The Van den Bergh reaction was of the biphasic prompt, direct type. Five days before death the icterus index was 110 units. The blood Wassermann and Kahn reactions were negative. The urine was acid in reaction, the specific gravity varying from 1.017 to 1.023. The Gmelin test for bilirubin was strongly positive. The urobilinogen in the urine was increased in amount, on one occasion being 4.3 mgm. per day, a week later 27.4 mgm. per day. The method used was a modification of one previously described by the writer (13). The upper limit of normal with this method is believed to be 1.5 mgm. per day. Over a four day period at about the time of the second urine urobilinogen estimation, the average daily excretion of urobilinogen in the feces was found to be 33 mgm. The normal range is from 100 to 250 mgm. per day, using the method just mentioned. These findings indicated an incomplete obstruction to the outflow of bile, and considerable diffuse liver injury. The stools did not contain demonstrable occult blood by the benzidine test.

Necropsy revealed advanced cirrhosis of the liver. The liver weighed 1125 grams, was quite firm and exhibited diffuse fine lobulation. The spleen weighed 300 grams. Microscopically a marked portal cirrhosis was seen in the liver, characterized by very marked fibrosis, moderate lymphocytic infiltration, and extensive bile duct proliferation in the portal spaces. Many of the bile ducts were dilated and filled with bile, as though definite obstruction existed distal to them. The liver parenchyma of the central portion of the lobules appeared to be quite normal. No necrosis was seen and fatty metamorphosis was relatively small in amount. Nothing from an anatomical standpoint served to distinguish this from an ordinary advanced portal cirrhosis.

The method used for the isolation of the coproporphyrin was a modification of that employed by H. Fischer and Duesberg (5). Each 24-hour urine sample for an eight day period was strongly acidified with glacial acetic

acid in a 5 liter separatory funnel, and shaken with  $\frac{1}{2}$  to  $\frac{3}{4}$  its volume of ether. (Care was observed at first during this shaking, because of the large amounts of  $\text{CO}_2$  liberated on acidification.) Emulsions were broken by addition of small amounts of alcohol. It was occasionally necessary to resort to filtration after shaking with talc. The ether was repeatedly washed with distilled  $\text{H}_2\text{O}$  and was next extracted a number of times with small amounts of 2 per cent HCl. This 2 per cent HCl from each 24-hour urine was united, and extracted repeatedly with chloroform, which removed urobilin and mesobiliviolin (14) as well as most of a small amount of chloroform soluble porphyrin which was present. It was then diluted ten times (to 0.2 per cent HCl) and again extracted with chloroform. This completed the removal of the chloroform soluble porphyrin. This porphyrin was probably protoporphyrin since on later fractionation it was found to leave 2 per cent HCl for chloroform. Nevertheless, its absorption spectrum did not correspond entirely with that of protoporphyrin, but the amount was much too small to permit further purification, so that its identity remained doubtful. The remaining aqueous solution was made negative to congo paper by addition of an excess of solid sodium acetate. Twenty to 25 cc. more of glacial acetic acid were also added. Repeated ether extraction was then carried out. The ether was united, washed with water repeatedly, and the coproporphyrin extracted with 2 per cent HCl. After nearly neutralizing with 10 per cent NaOH, it was again taken into ether. Next it was quantitatively removed from the ether with 10 per cent NaOH. This was allowed to stand over night, but the sodium salt was entirely soluble, as H. Fischer and Kirmann (15) have shown to be true of coproporphyrin, but not of the porphyrins more closely related to hemin, such as protoporphyrin, or deuteroporphyrin. Upon making this NaOH solution barely acid with 10 per cent HCl, the coproporphyrin was again taken into ether, and the 2 per cent HCl-ether fractionation was once more repeated. The final ether solution was dried over anhydrous sodium sulphate, filtered, and concentrated to a very small volume on the water bath. The free coproporphyrin crystallized in fine needles. This material was esterified by standing over night in methyl alcohol, which had just previously been saturated in the cold with dry HCl gas. The ester was purified, and crystallized out of chloroform-methyl alcohol, according to H. Fischer's method (4, 16). After repeated recrystallization, 12 mgm. of material were obtained. In handling such small amounts the use of a microtechnic is obligatory. This is particularly true with reference to filtration: funnels of 1.5 to 2 cm. in diameter with very small beaded glass ticks and round filter papers 4 to 5 mm. in diameter are employed with suction. A test tube with a side arm serves to receive the mother liquor. In this way a fraction of a milligram may readily be recrystallized several times. On standing, the separation of crystals out of chloroform-methyl alcohol is almost quantitative. After separation of a small amount of the ester

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Evidence for a dualism of the porphyrins in nature has been repeatedly presented by Hans Fischer (1, 2, 3). This means that both plant and animal organisms are capable of the formation of porphyrins of two isomeric series, those corresponding to aetioporphyrin III, including hemoglobin and chlorophyll, and those of the aetioporphyrin I type, the function of whose representatives is as yet very little understood. The aetioporphyrins, of which there are four, have only methyl and ethyl groups in varying position on the porphyrin ring. Because of this relative simplicity Fischer has classified the isomers of the other porphyrins according to which aetioporphyrin their structure corresponds. The aetioporphyrins are artificial. Porphyrins corresponding in structure to aetioporphyrins I and III have been found in nature, those corresponding to II and IV have only been obtained artificially. Fischer has pointed out that it is chemically inconceivable for porphyrins of the one type to be transformed into those of the other except by complete destruction and re-synthesis. Thus it is necessary to look upon the formation of the two series occurring in nature as independent processes. Porphyrins corresponding to aetioporphyrin I were first isolated from the urine and feces of a case of congenital porphyrinuria by H. Fischer (4). These were named coproporphyrin I and uroporphyrin I, the former having four methyl and four propionic acid groups, the latter eight carboxyl groups. Several instances of the same disease have since been studied by Fischer and Duesberg (5), and also Van den Bergh (6), in which identical porphyrins were excreted. However, one instance was reported by each in which coproporphyrin III was isolated. Thus evidence for the existence of two chemically different, but

clinically indistinguishable types of idiopathic porphyrinuria was presented. In only one other condition has coproporphyrin III been isolated: namely, by Grotepass (7) from the urine of a patient with lead poisoning. This finding was substantiated by Fischer and Duesberg (5) in a study of experimental lead poisoning in rabbits. Traces of coproporphyrin occur in normal urine, as recognized by Schumm (8), also by H. Fischer and Zerweck (9), but whether of the aetioporphyrin I or III type has not been determined. As Kämmerer (10) has recently observed, this remains one of the chief difficulties in the porphyrin problem, and the extremely small amounts in the normal urine make this obstacle almost insurmountable. H. Fischer (4) estimated that at least 1000 liters of normal urine would have to be employed in order to isolate sufficient porphyrin for microanalysis. The increase of porphyrin in the urine in jaundice and liver disease of various types has long been recognized (Garrod (11), Günther (12)). Until now porphyrins have not been isolated from such instances. In the present investigation a porphyrin has been isolated from the urine of a patient suffering from cirrhosis of the liver, probably caused by cincophen. This porphyrin has been identified by virtue of ester melting point and spectroscopic characteristics as coproporphyrin I.

### MATERIAL AND METHODS

A description of the clinical and anatomical findings in the patient from whose urine coproporphyrin I was isolated, follows:.

White male, aged 52. The patient came to the hospital because of jaundice and anorexia of eight weeks duration. For two years he had been troubled by rheumatism. To alleviate this he had taken a patent medicine for three months prior to the onset of jaundice. Chemical examination of this medicine indicated the presence of considerable amounts of cincophen. The jaundice

<sup>1</sup> Aided by a grant from research funds of the Graduate School of the University of Minnesota.

was painless. The stools were clay colored and the urine dark brown in color. There was marked ascites which recurred rapidly following each abdominal paracentesis. There was increasing edema of the legs. Hemorrhoids were noted. A definite hemorrhagic tendency was observed, characterized by petechiae and epistaxis. Neither liver nor spleen was palpable. The patient was apathetic, and during the last few days of life was difficult to arouse. Death occurred after a period of coma of about 36 hours duration. *Laboratory examinations:* The hemoglobin was 88 per cent (Sahli). The red blood cells numbered 4,500,000. Another hemoglobin determination one week before death was 96 per cent (Sahli). The leukocytes were 13,500, on another occasion 12,500; the neutrophils 84 per cent and the lymphocytes 16 per cent. The total leukocyte count one week before death had decreased to 7,800. The blood platelets were 200,000 per cu. mm. The coagulation time of blood obtained by ear lobe puncture was  $1\frac{1}{2}$  minutes; the bleeding time was 5 minutes. The capillary resistance test was strongly positive. The icterus index shortly after admission to the hospital was 140 units. The Van den Bergh reaction was of the biphasic prompt, direct type. Five days before death the icterus index was 110 units. The blood Wassermann and Kahn reactions were negative. The urine was acid in reaction, the specific gravity varying from 1.017 to 1.023. The Gmelin test for bilirubin was strongly positive. The urobilinogen in the urine was increased in amount, on one occasion being 4.3 mgm. per day, a week later 27.4 mgm. per day. The method used was a modification of one previously described by the writer (13). The upper limit of normal with this method is believed to be 1.5 mgm. per day. Over a four day period at about the time of the second urine urobilinogen estimation, the average daily excretion of urobilinogen in the feces was found to be 33 mgm. The normal range is from 100 to 250 mgm. per day, using the method just mentioned. These findings indicated an incomplete obstruction to the outflow of bile, and considerable diffuse liver injury. The stools did not contain demonstrable occult blood by the benzidine test.

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acid in a 5 liter separatory funnel, and shaken with  $\frac{1}{2}$  to  $\frac{3}{4}$  its volume of ether. (Care was observed at first during this shaking, because of the large amounts of  $\text{CO}_2$  liberated on acidification.) Emulsions were broken by addition of small amounts of alcohol. It was occasionally necessary to resort to filtration after shaking with talc. The ether was repeatedly washed with distilled  $\text{H}_2\text{O}$  and was next extracted a number of times with small amounts of 2 per cent HCl. This 2 per cent HCl from each 24-hour urine was united, and extracted repeatedly with chloroform, which removed urobilin and mesobiliviolin (14) as well as most of a small amount of chloroform soluble porphyrin which was present. It was then diluted ten times (to 0.2 per cent HCl) and again extracted with chloroform. This completed the removal of the chloroform soluble porphyrin. This porphyrin was probably protoporphyrin since on later fractionation it was found to leave 2 per cent HCl for chloroform. Nevertheless, its absorption spectrum did not correspond entirely with that of protoporphyrin, but the amount was much too small to permit further purification, so that its identity remained doubtful. The remaining aqueous solution was made negative to congo paper by addition of an excess of solid sodium acetate. Twenty to 25 cc. more of glacial acetic acid were also added. Repeated ether extraction was then carried out. The ether was united, washed with water repeatedly, and the coproporphyrin extracted with 2 per cent HCl. After nearly neutralizing with 10 per cent NaOH, it was again taken into ether. Next it was quantitatively removed from the ether with 10 per cent NaOH. This was allowed to stand over night, but the sodium salt was entirely soluble, as H. Fischer and Kirrmann (15) have shown to be true of coproporphyrin, but not of the porphyrins more closely related to hemin, such as protoporphyrin, or deuteroporphyrin. Upon making this NaOH solution barely acid with 10 per cent HCl, the coproporphyrin was again taken into ether, and the 2 per cent HCl-ether fractionation was once more repeated. The final ether solution was dried over anhydrous sodium sulphate, filtered, and concentrated to a very small volume on the water bath. The free coproporphyrin crystallized in fine needles. This material was esterified by standing over night in methyl alcohol, which had just previously been saturated in the cold with dry HCl gas. The ester was purified, and crystallized out of chloroform-methyl alcohol, according to H. Fischer's method (4, 16). After repeated recrystallization, 12 mgm. of material were obtained. In handling such small amounts the use of a microtechnic is obligatory. This is particularly true with reference to filtration: funnels of 1.5 to 2 cm. in diameter with very small bore glass tacks and round filter papers 4 to 5 mm. in diameter are employed with suction. A test tube with a side arm serves to receive the mother liquor. In this way a fraction of a milligram may readily be recrystallized several times. On standing, the separation of crystalline coproporphyrin-methyl ester from the mother liquor is complete. After separation of a small amount of the ester, the

obtained by allowing it to stand dissolved in 25 per cent HCl over night, sufficient sodium acetate was added to make the solution negative to congo paper, and the free porphyrin was taken into ether. This acetic and ether solution served for a spectrometric study of the porphyrin. For this purpose a Zeiss grating comparison spectrometer was employed.

In the above described separation of the porphyrins, a more complete fractionation was effected by continuing the extractions until the absence or extreme faintness of the red fluorescence in ultra-violet light, of the porphyrin being extracted, indicated that the extraction was complete. This red fluorescence persists long after the porphyrin color and absorption spectrum has ceased to be visible. As a source of ultra-violet light, a small Leitz hand arc lamp was used, a copper sulfate solution being employed to filter out a considerable part of the light of longer wave length.

### RESULTS

The crystals of the porphyrin ester obtained have the appearance shown in Figure 1. The



FIG. 1. CRYSTALS OF THE PORPHYRIN ESTER FROM THE URINE.

methyl ester of coproporphyrin I crystallizes characteristically in this form, i.e., thread like needles; while the crystals of coproporphyrin III methyl ester, as recently compared by H. Fischer and Duesberg (5), are less needle like, having the form of broader prisms. The porphyrin ester isolated in the above procedure, after three recrystallizations from chloroform-methyl alcohol, melted at 241 to 243° C. Coproporphyrin I methyl ester (for which the writer is indebted to Professor H. Fischer) melted at 245 to 246° C. A mixture showed no significant depression of melting point, which was 243 to 245° C. The absorption spectrum of the acetic and ether solu-

tion of the free porphyrin was as follows: I, 625.4 m $\mu$  to 621.4, maximum 623.5 m $\mu$ , II, very faint, maximum 596.8 m $\mu$ , III, 569.0 m $\mu$  to 567.1, maximum 568.2 m $\mu$ , IV, 532.8 m $\mu$  to 524.2, maximum 529.1 m $\mu$ , V, 505.2 m $\mu$  to 488.1, maximum 495.1 m $\mu$ . The order of intensity was: V, IV, I, III, II. This solution exhibited identical absorption with that of a similarly prepared solution of coproporphyrin I, the spectra of the two solutions being superimposed in the comparison spectrometer.

### DISCUSSION

There was no suggestion in the behavior or appearance of the patient's erythrocytes, during life, of bone marrow irritation. Judging by Weir and Comfort's (17) recent review of the reported cases of cincophen poisoning, anemia or other evidence of marrow affection is extremely rare. There is little reason, therefore, to assume that this increase of coproporphyrin in the urine occurred because of increased formation in the marrow such as is the case in congenital porphyrinuria and pernicious anemia (H. Fischer, H. Hilmer *et al.* (18)). Rather than derived from excessive formation in the marrow it was believed that the porphyrin isolated was the normal coproporphyrin of the urine, increased because of the damaged excretory power of the liver. Van den Bergh (19) has recently discussed the retention of coproporphyrin in the blood serum in obstructive jaundice. He obtained experimental evidence to suggest that the coproporphyrin of the bile is formed in the liver. According to this, one would expect relatively large amounts of urinary coproporphyrin in simple obstructive jaundice, and smaller amounts or none in jaundice due to liver disease. This was apparently not true in the present case, since there was, of course, advanced liver disease. Nevertheless, the damage was fairly well limited to the periphery of the lobules, and there was obviously a definite biliary obstruction. In three cases of common duct obstruction due to stone, the urine was found to contain definite, but only moderate increases of coproporphyrin, very much less in amount than in the case of cincophen cirrhosis. On the other hand, the urine from a patient who later died of hepatic insufficiency due to advanced hepar lobatum contained no trace of either copro- or any other porphyrin.

## SUMMARY

A porphyrin has been isolated from the urine of a patient with cirrhosis of the liver, the latter probably caused by cincophen. So far as can be determined this is the first report of the isolation of a porphyrin from the urine of a patient suffering from jaundice, or liver disease. It is believed to be the normal coproporphyrin of the urine, increased in amount because of damaged excretory power of the liver. It has been positively identified by virtue of ester melting point and spectroscopic character as coproporphyrin I.

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obtained by allowing it to stand dissolved in 25 per cent HCl over night, sufficient sodium acetate was added to make the solution negative to congo paper, and the free porphyrin was taken into ether. This acetic and ether solution served for a spectrometric study of the porphyrin. For this purpose a Zeiss grating comparison spectrometer was employed.

In the above described separation of the porphyrins, a more complete fractionation was effected by continuing the extractions until the absence or extreme faintness of the red fluorescence in ultra-violet light, of the porphyrin being extracted, indicated that the extraction was complete. This red fluorescence persists long after the porphyrin color and absorption spectrum has ceased to be visible. As a source of ultra-violet light, a small Leitz hand arc lamp was used, a copper sulfate solution being employed to filter out a considerable part of the light of longer wave length.

### RESULTS

The crystals of the porphyrin ester obtained have the appearance shown in Figure 1. The



FIG. 1. CRYSTALS OF THE PORPHYRIN ESTER FROM THE URINE.

methyl ester of coproporphyrin I crystallizes characteristically in this form, i.e., thread like needles; while the crystals of coproporphyrin III methyl ester, as recently compared by H. Fischer and Duesberg (5), are less needle like, having the form of broader prisms. The porphyrin ester isolated in the above procedure, after three recrystallizations from chloroform-methyl alcohol, melted at 241 to 243° C. Coproporphyrin I methyl ester (for which the writer is indebted to Professor H. Fischer) melted at 245 to 246° C. A mixture showed no significant depression of melting point, which was 243 to 245° C. The absorption spectrum of the acetic and ether solu-

tion of the free porphyrin was as follows: I, 625.4  $m\mu$  to 621.4, maximum 623.5  $m\mu$ , II, very faint, maximum 596.8  $m\mu$ , III, 569.0  $m\mu$  to 567.1, maximum 568.2  $m\mu$ , IV, 532.8  $m\mu$  to 524.2, maximum 529.1  $m\mu$ , V, 505.2  $m\mu$  to 488.1, maximum 495.1  $m\mu$ . The order of intensity was: V, IV, I, III, II. This solution exhibited identical absorption with that of a similarly prepared solution of coproporphyrin I, the spectra of the two solutions being superimposed in the comparison spectrometer.

### DISCUSSION

There was no suggestion in the behavior or appearance of the patient's erythrocytes, during life, of bone marrow irritation. Judging by Weir and Comfort's (17) recent review of the reported cases of cincophen poisoning, anemia or other evidence of marrow affection is extremely rare. There is little reason, therefore, to assume that this increase of coproporphyrin in the urine occurred because of increased formation in the marrow such as is the case in congenital porphyrinuria and pernicious anemia (H. Fischer, H. Hilmer *et al.* (18)). Rather than derived from excessive formation in the marrow it was believed that the porphyrin isolated was the normal coproporphyrin of the urine, increased because of the damaged excretory power of the liver. Van den Bergh (19) has recently discussed the retention of coproporphyrin in the blood serum in obstructive jaundice. He obtained experimental evidence to suggest that the coproporphyrin of the bile is formed in the liver. According to this, one would expect relatively large amounts of urinary coproporphyrin in simple obstructive jaundice, and smaller amounts or none in jaundice due to liver disease. This was apparently not true in the present case, since there was, of course, advanced liver disease. Nevertheless, the damage was fairly well limited to the periphery of the lobules, and there was obviously a definite biliary obstruction. In three cases of common duct obstruction due to stone, the urine was found to contain definite, but only moderate increases of coproporphyrin, very much less in amount than in the case of cincophen cirrhosis. On the other hand, the urine from a patient who later died of hepatic insufficiency due to advanced hepar lobatum contained no trace of either copro- or any other porphyrin.

## SUMMARY

A porphyrin has been isolated from the urine of a patient with cirrhosis of the liver, the latter probably caused by cincophen. So far as can be determined this is the first report of the isolation of a porphyrin from the urine of a patient suffering from jaundice, or liver disease. It is believed to be the normal coproporphyrin of the urine, increased in amount because of damaged excretory power of the liver. It has been positively identified by virtue of ester melting point and spectroscopic character as coproporphyrin I.

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# CONCERNING THE NATURALLY OCCURRING PORPHYRINS

## II. THE ISOLATION OF A HITHERTO UNDESCRIBED PORPHYRIN OCCURRING WITH AN INCREASED AMOUNT OF COPROPORPHYRIN I IN THE FECES OF A CASE OF FAMILIAL HEMOLYTIC JAUNDICE<sup>1</sup>

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Van den Bergh (19) has recently obtained experimental evidence to suggest that coproporphyrin may be formed in the liver from protoporphyrin. Having a short time previously described the regular occurrence of protoporphyrin in human erythrocytes (20) he pointed out the possibility that the coproporphyrin of human bile may have its origin in this way, i.e., protoporphyrin liberated from the red blood cells and transformed into coproporphyrin in the liver. The protoporphyrin of the erythrocytes has as yet received relatively little study. Van den Bergh's observation as to its occurrence has been confirmed by Kämmerer (10), and by Schreus (21). It has been assumed by Van den Bergh to correspond to aetioporphyria III, in other words, to be related either to the formation or destruction of hemoglobin. Mention has been made in part I of the dualism of the porphyrins which H. Fischer has shown to exist in nature. A most complete review of this subject in its relation to physiology and medicine will be found in Kämmerer's presentation (10) before the German Congress of Internal Medicine in 1933.

If the coproporphyrin in the bile comes from the protoporphyrin of the erythrocytes, a considerable increase in its amount in both bile and feces might be expected during a period of increased blood destruction. Furthermore, if the protoporphyrin giving rise to coproporphyrin in the liver, does correspond to aetioporphyria III, then the increased coproporphyrin excreted should be coproporphyrin III. For this reason the writer has examined the feces as to porphyrin content in five cases of congenital hemolytic jaundice. One of these was of particular interest since the patient was under observation during a

so-called hemolytic crisis. The porphyrin excretion in the feces during this period was actually greatly increased. In the following, particular emphasis will be given to the description of the porphyrins isolated from the feces of this case.

Because of Boas' (22) recent description of the occurrence of protoporphyrin in the urine and his suggestion that it might be found in urines of individuals having increased blood destruction, the urine from this case was also carefully examined as to porphyrin content. Günther (12), later H. Fischer and Zerweck (9), and R. Duesberg (23) examined the urine in cases of hemolytic jaundice without being able to demonstrate any increase in porphyrin content.

### MATERIAL AND METHODS

*Case 1.* Male, aged 22. The patient stated that he had never been strong and that for many years he had been subject to attacks of short duration characterized by nausea, vomiting, and fever. In the past three years jaundice had been noted repeatedly and anemia had been at times severe enough to require transfusion. The patient's mother believed that jaundice and anemia had been present in early childhood. The patient was first examined on November 21, 1933. At this time jaundice was moderate, and there appeared to be some anemia. He complained of weakness and tiredness; the mouth temperature was elevated to 99.4° F. The spleen was markedly enlarged, reaching to about the level of the umbilicus. Its consistency was firm. The hemoglobin percentage on this date was 54 (Sahli). The erythrocytes were 2,570,000; leukocytes were normal. Stained smears of the blood revealed marked anisocytosis with many hyperchromatic microcytes. The reticulated erythrocytes were 37 per cent. The icterus index was 20. The

<sup>1</sup> Aided by a grant from research funds of the Graduate School of the University of Minnesota.

Van den Bergh reaction was of the indirect type. Resistance of the patient's erythrocytes to hypotonic saline was as follows: hemolysis began at 0.66 per cent, was complete at 0.44 per cent. Hemolysis of control erythrocytes began at 0.44 per cent, was complete at 0.34 per cent. None of the members of the patient's immediate family, including his parents and several brothers, gave any history of jaundice, nor was the spleen palpable in any. Nevertheless, the erythrocytes from the father and one brother were tested as to fragility with interesting result. Father's erythrocytes: hemolysis began at 0.42 per cent and was complete at 0.34 per cent. Hemolysis of the brother's erythrocytes began at 0.54 per cent and was complete at 0.38 per cent. Control: Hemolysis began at 0.44 per cent, was complete at 0.32 per cent. The urobilinogen excretion in the feces was estimated over a four day period, November 21 to November 25. The average daily amount was 1140 mgm. The normal range with the method (13) used is from 100 to 250 mgm. per day. Preparatory to splenectomy the patient was given a transfusion of 600 cc. of citrated blood on November 26. It should be stated that even before the transfusion the patient complained of weakness and anorexia and the temperature was elevated to 100° F. There was no immediate reaction to transfusion. On the following day the patient felt much worse, the temperature rose to 103°, nausea, restlessness, and apprehension were noted. The jaundice had increased. On November 29 the icterus index had increased to 32, the hemoglobin percentage had decreased to 45 (Sahli). The average daily excretion of urobilinogen in the feces from November 28 to December 2 was 1220 mgm. It was believed that the patient was suffering from a hemolytic crisis, and that the transfusion rather than having been of aid had perhaps precipitated this increase of blood destruction. Following this temporary marked increase of jaundice and anemia, improvement was rapid. The temperature was normal on December 1 and the patient felt much better. After three days he was allowed to go home and returned on December 12. At this time he had improved remarkably. The icterus index was 20, jaundice definitely diminished. The hemoglobin percentage was now 78 (Sahli), and the erythrocytes were 4,200,000 per c.mm. The

reticulocytes had decreased to 9 per cent. Splenectomy was done by Dr. O. H. Wangenstein. The spleen weighed 1720 grams. The capsule was smooth, the external surface was dark red, the consistency firm. The cut section was dark red, and it was apparent that the pulp contained a large amount of blood. Microscopic sections exhibited the usual appearance of spleens from cases of familial hemolytic icterus, i.e., narrow sinuses, the splenic pulp crowded with erythrocytes. Relatively little iron was demonstrable with the Berlin blue reaction. The spleen was not examined as to porphyrin content. This had previously been done in a similar instance (24) with negative result.

The collection of feces from which porphyrins were isolated covered two periods, November 21 to November 25, and November 28 to December 2, eight days in all. The moist feces for this period weighed 920 grams. Twenty grams were used in the quantitative urobilinogen estimations. One gram of the mixed collection was used for a benzidine test for occult blood, which was negative.

All of the remainder was thoroughly ground in a large mortar with glacial acetic acid. This mixture was repeatedly extracted with relatively larger amounts of ether, in the same way as recently described (25) for the isolation of crystalline stercobilin from feces. The acetic ether extract was filtered and the ether largely removed by vacuum distillation. The remaining acetic acid solution was poured into 6 volumes of 2 per cent HCl, with thorough mixing. After standing overnight this was filtered, and the filtrate in a five liter separatory funnel was covered with approximately 500 cc. of ether. Solid sodium acetate was now added in sufficient amount to make the aqueous fraction negative to congo paper. The two fractions were thoroughly shaken immediately after each addition of sodium acetate. After making the solution congo negative, it was shaken out four more times with smaller amounts of ether. The entire ether solution, containing all of the porphyrins, a considerable proportion of the copromesobiliviolin (26), and a relatively small proportion of the stercobilin, was washed repeatedly with distilled water, which removed most of the latter. The porphyrins and the copromesobiliviolin were removed by repeated ex-

traction with 2 per cent HCl. The copromesobiliviolin was removed from this by repeated extraction with chloroform. The porphyrins, with the exception of protoporphyrin, remain in the 2 per cent HCl.

The 2 per cent HCl solution was diluted with distilled water to 0.2 per cent. Following this dilution chloroform extraction was again resorted to. H. Fischer (27) was able to show that coproporphyrin may be separated from deuteroporphyrin by repeatedly extracting a 0.2 per cent HCl solution of the two porphyrins with chloroform. Only the deuteroporphyrin goes into the chloroform. This method was carried out by the writer (24) in isolating deuteroporphyrin III from the feces.

The chloroform solution had a wine red color and exhibited intense red fluorescence in ultra-violet light, indicating a considerable porphyrin content. It was shaken repeatedly in a separatory funnel with 10 per cent NaOH, the porphyrin leaving the chloroform quantitatively as a difficultly soluble sodium salt. On standing over night, most of this came out of solution. It was collected on a filter paper, washed with a very little 10 per cent NaOH, and then redissolved in 10 per cent HCl. It was then taken into ether by repeated extraction, the HCl being almost neutralized by the gradual addition of 10 per cent NaOH. The ether solution was dried over anhydrous sodium sulphate, filtered and evaporated to dryness on the water bath. The partially crystalline residue was dissolved in methyl alcohol saturated in the cold with dry HCl gas. The purified ester was crystallized out of chloroform-methyl alcohol. All of these procedures were carried out in accordance with H. Fischer's (4, 16) methods. After three recrystallizations, the yield was approximately 7 mgm. As will be seen below, this was found to be a hitherto undescribed porphyrin.

The porphyrin remaining in the 0.2 per cent HCl was taken into ether after making the solution negative to congo paper by the addition of sodium acetate and more strongly acid by the addition of glacial acetic acid. After washing the ether several times with distilled water, the porphyrin was extracted with 10 per cent NaOH. The sodium salt of this porphyrin was soluble. The solution was filtered and the porphyrin taken

into ether by barely acidifying with 10 per cent HCl and shaking in a separatory funnel. The ether was washed with water and the porphyrin quantitatively extracted with 1 per cent HCl. This fractionation: ether—1 per cent HCl—ether was repeated, just neutralizing the acid each time by dropping in 10 per cent NaOH. The ether was dried over anhydrous sodium sulphate. Upon concentrating it to a very small volume on the water bath, the free porphyrin crystallized in fine needles. The small amount of solution was filtered and all of the crystalline material including that on the walls of the round bottomed flask was esterified as described above. The ester crystallized readily from chloroform-methyl alcohol. The yield was approximately 6 mgm. As will be described below this was found to be the methyl ester of coproporphyrin I.

For the purpose of spectroscopic study small amounts of the esters were saponified by standing over night in 25 per cent HCl. This was made negative to congo paper, and the free porphyrins extracted with ether. Measurements of absorption spectra were made with the aid of a Zeiss grating comparison spectrometer. Comparisons of spectra of known and newly isolated porphyrins were made by superimposing the spectra of acetic and ether solutions of the two in this apparatus. In this way it is possible to make certain of very small differences in position of absorption bands. These differences are often too small to be recognized with a hand spectroscope.

Coproporphyrin was demonstrated in the urine of Case 1 by employing the acetic and ether extraction method as used for the isolation of coproporphyrin I from the urine, described in Part I. The amount obtained from a 24-hour urine sample was too small to color more than faintly the final solution in 2 per cent HCl, 20 cc. in volume. This, however, exhibited a fairly intense red fluorescence in ultra-violet light. The amount of porphyrin was too small to permit of spectroscopic identification. It was ether soluble and did not leave 0.2 per cent HCl for chloroform, characteristics which identify it fairly well as a coproporphyrin. Two months after splenectomy feces from Case 1 for an eight day period of collection, were again examined, using the method just described. At this time there was no jaundice nor anemia and the patient felt very much improved.

Feces for eight day periods of collection from four other typical cases of familial hemolytic anemia were also investigated as to porphyrin content. The method described above for isolating porphyrins from the feces was used in each instance. In three of the cases (3, 4, and 5), no complicating disease was present. In one (Case 2), chronic ulcerative colitis had been recognized for two years; during the period of collection of feces the patient was having from four to six stools per day. These at times contained traces of blood. In none of these four cases was there any evidence of a hemolytic crisis such as occurred in Case 1. In this study, no attempt has been made to estimate the amounts of porphyrin excreted, principally because no accurate and satisfactory method is yet available. Nevertheless, it was possible to gain what was believed to be a fairly accurate conception of amount by the amounts which could be isolated, as well as by comparisons of the intensity of color and absorption spectrum of the final solutions from amounts of feces representing the same periods of collection. The cases used for comparison will be mentioned in Part III of this communication.

#### RESULTS

The methyl ester of the chloroform soluble porphyrin obtained from the feces of Case 1 crystallized in flower-like aggregates of well formed, somewhat curved prisms (Figure 1). The ease of crystallization, as well as the unity of the crystal form spoke against the possibility of a mixture.

This ester melted sharply at 202 to 203° C. There was no elevation of the melting point after another recrystallization. The dimethyl ester of deuteroporphyrin III melts at 223°, that of mesoporphyrin IX at 212°, that of hematoporphyrin at 212° (H. Fischer (2)). Mixed melting point determinations with deuteroporphyrin III and mesoporphyrin IX ester, the latter obtained from hemin according to H. Fischer and Kögl's method (28), gave sharp depressions.

The "HCl number" (2) of the new porphyrin was found to be 0.8 per cent. This indicates the lowest concentration of HCl which will remove the porphyrin from ether. The absorption spectrum of this porphyrin in ether and acetic was: I 630.1 to 621.9  $m\mu$ , maximum 627.4  $m\mu$ , II (very



FIG. 1. CRYSTALS OF PORPHYRIN ESTER FROM THE FECES IN CASE 1.

weak) 597.2  $m\mu$ , maximum, III 582.8 to 566.7  $m\mu$ , maximum 572.4  $m\mu$ , IV 536.4 to 525.0  $m\mu$ , maximum 531.4  $m\mu$ , V 506.6 to 486.4  $m\mu$ , maximum 496.9  $m\mu$ . Order of intensity: V, IV, III, I, II. A small amount of this porphyrin was submitted to Professor Hans Fischer in Munich, to whom the writer is most grateful for suggestions and advice regarding it. Professor Fischer found: I 628.9  $m\mu$ , maximum, II 572.3  $m\mu$ , III 532.9  $m\mu$ , IV 499.4  $m\mu$ ; order of intensity IV, III, II, I. He observed that this was not characteristic either of hematoporphyrin or deuteroporphyrin, but more like a mixture of protoporphyrin and hematoporphyrin. Because of this he pointed to the possibility of a molecular combination of the two. Another possibility which he suggested was that of a protoporphyrin changed to the extent of having a molecule of water added on to one of its vinyl ( $\text{CH}=\text{CH}_2$ ) groups. Hematoporphyrin in concentrated sulphuric acid exhibits a marked spectroscopic change compared with the original substance; deuteroporphyrin shows no change (27). The writer did not observe any change in concentrated sulphuric acid after one hour. However, Professor Fischer noted a definite although slight shift of absorption after the solution had stood for three days. His spectroscopic findings were: I 597.5  $m\mu$ , II 552.8  $m\mu$ . After three days I 596.5  $m\mu$ , II 558.8  $m\mu$ . Deuteroporphyrin is readily brominated giving dibromodeuteroporphyrin with considerable change in spectroscopic character, while mesoporphyrin is unchanged by bromine. The new porphyrin exhibited a very definite change with bromine, and

the absorption was now identical with that of dibromodeuteroporphyrin III. The effect of bromine was observed with the free porphyrin dissolved in glacial acetic acid. Deuteroporphyrins corresponding to aetioporphyrin I have not been synthesized (27), so that no reference substance is available to aid in determining whether the new porphyrin is an isomeric deuteroporphyrin, or an entirely new type of porphyrin. Microanalysis would be of great help in classifying this porphyrin. To this end more material is necessary, and feces from more individuals exhibiting a marked increase of blood destruction must be studied as to porphyrin content. The feces from Case 1 obtained two months after splenectomy contained only a trace of chloroform soluble porphyrin. The amount was too small to be identified spectroscopically.

In three of the four other cases studied (2, 3, and 4), the amounts of chloroform soluble porphyrin were not sufficient for identification. From the feces of Case 5, however, a chloroform soluble porphyrin was obtained in sufficient concentration to permit spectroscopic study. In ether and acetic the absorption spectrum was: I 630.8 to 621.8  $m\mu$ , maximum 627.4  $m\mu$ , II 582.8 to 567.3  $m\mu$ , maximum 576.2  $m\mu$ , III 538.6 to 526.1  $m\mu$ , maximum 533.0, IV 509.2 to 491.7  $m\mu$ , maximum 498.3  $m\mu$ . Order of intensity: IV, III, I, II. Superimposed with the new porphyrin from Case 1, the absorption spectra were identical. Thus the occurrence of the newly described porphyrin in the feces of another case of familial hemolytic jaundice, while not proven, is strongly suggested.

The ester of the porphyrin remaining in 0.2 per cent HCl, from Case 1, crystallized in the form of thread like needles, characteristic of coproporphyrin I ester (see Figure 1 in Part I). This melted at 245 to 246° C. The melting point of coproporphyrin I methyl ester was identical. A mixture gave no depression of the melting point. In acetic and ether the absorption spectrum was: I 625.4 to 621.4  $m\mu$ , maximum 623.5  $m\mu$ , II (very faint) maximum at 596.8  $m\mu$ , III 569.0 to 567.1  $m\mu$ , maximum 568.2  $m\mu$ , IV 532.8 to 524.2  $m\mu$ , maximum 529.1  $m\mu$ , V 505.2 to 488.1  $m\mu$ , maximum 495.1  $m\mu$ . Order of intensity: V, IV, I, III, II. Spectroscopic identity with copropor-

phyrin I was demonstrated, the spectra being superimposed.

Roughly estimated, the per diem amount of coproporphyrin I excreted by Case 1, before splenectomy, was at least 20 to 30 times that normally observed. This was much more than was found in the other four cases, although the amount of coproporphyrin in the feces of each of these was definitely greater than from normal individuals and individuals with secondary anemias. The cases used for comparison will be mentioned in Part III of this communication. Coproporphyrin I was readily isolated from the feces of Cases 3 and 5; in the former the amount obtained after three recrystallizations was approximately 2 mgm., in the latter 3 mgm. The melting points in each instance were identical with that of coproporphyrin I, and no depression of the melting point of a mixture was observed. The absorption spectra in both instances were identical with that of coproporphyrin I, the spectra being superimposed. The amount of porphyrin from Case 4 was easily sufficient to permit crystallization, and was at least as much as in the last two instances, but it was unfortunately lost during recrystallization. The amount from Case 2 was not enough to be obtained in crystalline form, although the intensity of color and absorption spectrum indicated considerably more porphyrin than usually noted.

#### DISCUSSION

In only one way would it be possible to correlate the occurrence of excessive amounts of coproporphyrin I in the feces during heightened blood destruction with Van den Bergh's conception that the protoporphyrin of the erythrocytes is transformed in the liver into coproporphyrin. This would presuppose that this protoporphyrin also corresponds to aetioporphyrin I, in other words, that it has no direct relation to the formation or destruction of hemoglobin. The only protoporphyrin as yet isolated is that whose iron chloride compound is ordinary hemin, whose structure corresponds to aetioporphyrin III. Nevertheless, it is quite possible that isomeric protoporphyrins exist in nature, and that the formation of coproporphyrin I in the body proceeds over a protoporphyrin of the "I" type. H. Fischer and Kirrman (15) have shown that protoporphyrin may be con-



verted to coproporphyrin by addition of formic acid to the two vinyl groups of the former. These relationships have been thoroughly presented by Fischer in various communications (1, 2, 3).

Until the isomeric type of the new porphyrin can be determined, little can be said regarding its significance. Its characteristics classify it rather definitely as a closer relative of protoporphyrin than is coproporphyrin. If new evidence shall prove it to correspond to aetioporphyrin III, then its occurrence during heightened blood destruction could only be regarded as indicating biliary excretion of a porphyrin derived from hemoglobin. If it be found to correspond to aetioporphyrin I, its occurrence during increased blood destruction would strongly suggest that the erythrocytes contain a protoporphyrin corresponding to aetioporphyrin I.

The possibility that these porphyrins were formed in the bone marrow, not in the liver, must be considered. Against this view was the very small amount of coproporphyrin, and the total absence of a chloroform soluble porphyrin, in the urine of Case 1. A microspectroscopic study of the marrow in familial hemolytic jaundice such as Borst and Königsdörffer (29) have carried out

in pernicious anemia, would serve to support or deny the possibility of bone marrow formation.

#### SUMMARY

A hitherto undescribed porphyrin whose methyl ester melts at 202 to 203° C., having some of the characteristics of a deuteroporphyrin, but differing from the deuteroporphyrins spectroscopically, has been isolated from the feces of a typical case of familial hemolytic jaundice, suffering from a "hemolytic crisis" at the time the feces were collected. This porphyrin occurred in association with a marked increase of coproporphyrin I. In four other cases of the same disease the excretion of coproporphyrin was moderately increased, and in two of these instances it was again isolated and shown to be coproporphyrin I. If this coproporphyrin is to be related to the protoporphyrin of the erythrocytes, as described by Van den Bergh, the latter would have to correspond to aetioporphyrin I. The possibility of independent formation in the marrow erythroblasts must also be considered.

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# CONCERNING THE NATURALLY OCCURRING PORPHYRINS

## III. THE ISOLATION OF COPROPORPHYRIN I FROM THE FECES OF UNTREATED CASES OF PERNICIOUS ANEMIA<sup>1</sup>

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H. Fischer and Zerweck (9) were the first to recognize the increased urinary excretion of coproporphyrin in cases of pernicious anemia during relapse. Traces of coproporphyrin occur regularly in the normal urine and evidence was presented in Part I of this communication which strongly suggests that the normal urine porphyrin is coproporphyrin I. There is no evidence that this is formed in the bone marrow of the normal individual, rather, the studies of Borst and Königsdörffer (29) are against this view. Van den Bergh's experimental evidence suggesting that coproporphyrin may be formed in the liver has been discussed in Part II. Recently coproporphyrin has been identified in the erythroblasts and megaloblasts of pernicious anemia marrow by Borst and Königsdörffer (29). The reason for this formation of coproporphyrin in the marrow is unknown. Duesberg (23) has assumed it to be simply another evidence of reversion of the pernicious anemia marrow to an embryonic type, since the erythroblasts of normal embryonic marrow regularly contain coproporphyrin according to Borst and Königsdörffer (29), and since coproporphyrin is regularly present in meconium, Garrod (11), Günther (12). On the other hand, H. Fischer and Hilmer (30) were able to isolate coproporphyrin I from ordinary brewer's yeast, the amount being greatly increased after autolysis. It was conceivable that the marrow cells in pernicious anemia were to be regarded as cells laboring under abnormal or deficient living conditions, the coproporphyrin being formed for an unknown function in the same way that the yeast cell forms it under abnormal conditions. A further possibility was that the coproporphyrin excreted in pernicious anemia corresponded to aetioporphyria III, in other words, to hemoglobin, as Grotepass

(7) has recently shown to be the case in lead poisoning. Definite knowledge as to the type of coproporphyrin excreted in pernicious anemia is necessary. The amounts in the urine have been too small to permit of its isolation from that source. In 1932, the writer reported the isolation of a coproporphyrin from the feces of a pernicious anemia patient (24). The amount was so small that sufficient recrystallization was not possible; nevertheless, the ester melted at 220°, high enough to preclude the possibility of its being coproporphyrin III. In the present investigation, feces from normal individuals, from individuals suffering from secondary anemias, and from pernicious anemia patients were investigated as to their porphyrin content.

### MATERIAL AND METHODS

The method used for the isolation of the porphyrins from the feces was that described in Part II.

Feces from two cases of pernicious anemia were investigated. The clinical findings in these are briefly described as follows:

*Case 1.* Female, aged 51. The principal symptoms were weakness, sore tongue, and paresthesias. The stomach contents did not contain free HCl. The hemoglobin was 31 per cent (Sahli), erythrocytes 1,000,000, leukocytes 2,050, 64 per cent lymphocytes. Stained smears showed marked macro-anisocytosis, poikilocytosis, occasional basophilic stippling and multiple nuclear buds. Reticulocytes 2.4 per cent. Following intramuscular liver therapy the reticulocyte count increased to 12.1 per cent with progressive increase of hemoglobin and erythrocytes, and marked improvement in condition.

The entire amount of feces for an eight day period prior to liver therapy was subjected to the procedure described in Part II. The patient was seen again ten months later. The hemoglobin

<sup>1</sup> Aided by a grant from research funds of the Graduate School of the University of Minnesota.

was now 80 per cent (Sahli), the erythrocytes 3,840,000. The feces for a like period were again examined in the same way as to porphyrin content.

*Case 2.* Female, aged 60. Pale yellow skin, weakness, and sore tongue of six months duration. Hemoglobin 25 per cent, erythrocytes 900,000, reticulocytes 0.5 per cent, leukocytes 2,100, 74 per cent lymphocytes. Marked macro-anisocytosis, and poikilocytosis. Some basophilic stippling, occasional normoblasts. The entire amount of feces for six days was subjected to the method of isolation.

The feces from six individuals not having pernicious anemia were examined in the same way for the sake of comparison. Two were normal male adults. The collection of feces from both of these covered an eight day period. The other four were hospital patients. One had a high grade anemia secondary to a bleeding duodenal ulcer. The collection of feces from this case covered a period of nine days. Two of the patients had macrocytic anemias, one in association with cirrhosis of the liver, the other with tuberculous lymphadenitis and probable tuberculosis of the spleen. The feces from the former were collected for six days, those of the latter for eight. One patient had the typical symptoms and findings of chronic idiopathic hypochromic anemia, including sore tongue, dysphagia, hypochlorhydria, koilonychia, and color index 0.54. The period of collection of feces was eight days. All these individuals were on a general diet with exception of the patient with the bleeding ulcer, who received only milk.

Spectroscopic studies were made with a Zeiss spectrometer of grating type with a comparison prism permitting the absorption spectra of two solutions to be superimposed. For the purpose of spectroscopic study, small amounts of the esters of the porphyrins isolated were saponified in 25 per cent HCl. The absorption spectra of the free porphyrins were then measured, both in 25 per cent HCl, and in ether and acetic solution. They were also directly compared with those of known porphyrins, in the same solvents, by superimposition of their spectra.

## RESULTS

Coproporphyrin I was isolated in the form of its crystalline methyl ester from the feces of both of the cases of pernicious anemia. The amount obtained from the first case after three recrystallizations was approximately 1 mgm., from the second 0.6 mgm. The melting point of that from the first case was 245 to 247° C. and from the second 243 to 245° C. In neither was there any depression of the melting point of a mixture with known coproporphyrin I which melted at 245 to 246° C.

The absorption spectrum of the porphyrin from the first case in ether and acetic was as follows: I 624.5 m $\mu$  to 620.8, maximum 623.1 m $\mu$ , II maximum 566.9 m $\mu$ , III 531.1 m $\mu$  to 523.9, maximum 528.5 m $\mu$ , IV 505.0 m $\mu$  to 489.0, maximum 496.2 m $\mu$ . Order of intensity: IV, I, III, II.

The absorption spectrum of the second in ether and acetic was: I 624.6 m $\mu$  to 621.9, maximum 623.3 m $\mu$ , II faint maximum 567.8 m $\mu$ , III 532.4 m $\mu$  to 524.4, maximum 529.1 m $\mu$ , IV 505.6 m $\mu$  to 488.2, maximum 496.4 m $\mu$ . Order of intensity: IV, I, III, II. In 25 per cent HCl: I 596.4 m $\mu$  to 591.1, maximum 593.4 m $\mu$ , II faint maximum 573.0 m $\mu$ , III 557.5 m $\mu$  to 544.8, maximum 551.8 m $\mu$ . Order of intensity: III, I, II.

Feces from the first case, studied ten months after liver therapy had induced a remission, contained so very little coproporphyrin that it could not be obtained in crystalline form. A chloroform soluble porphyrin was present in small amount. This left 0.2 per cent HCl for chloroform, and gave an insoluble sodium salt, characteristics of a deuteroporphyrin. The amount after three recrystallizations was approximately 0.5 mgm. The crystals (Figure 1) of its methyl ester were like those of the ester of deuteroporphyrin III previously isolated (24). However, the melting point of these crystals was 189 to 191° C. Deuteroporphyrin III methyl ester melts at 221° C. A mixture melted at 186 to 189° C. Surprisingly enough, the absorption spectrum of this porphyrin was practically identical with that of coproporphyrin. In acetic and ether solution the absorption was as follows: I 624.9 m $\mu$  to 622.9, maximum 623.6 m $\mu$ , II faint maximum 609.1 m $\mu$ , III 589.8 m $\mu$  to 576.8, maximum 582.8 m $\mu$ .



FIG. 1. CRYSTALS OF THE PORPHYRIN ESTER FROM THE FECES IN CASE 1.

$m\mu$ , IV 533.7  $m\mu$  to 523.6, maximum 528.9  $m\mu$ , V 504.8  $m\mu$  to 484.9, maximum 496.1  $m\mu$ . Order of intensity: V, I, IV, III, II. The spectrum was further compared with that of the new porphyrin described in Part II, and found to differ. It is possible that this is the same porphyrin as that obtained repeatedly by H. Fischer and Duesberg (5) from the feces of rabbits. The ester melting point of the porphyrin described by them varied between 182° and 187° C. Its absorption spectrum was evidently more nearly that of a deuteroporphyrin than in the present instance, and the authors believed it to be either an impure deuteroporphyrin III, or an isomer. The same may be true of the porphyrin described above. In the absence of positive identification, nothing can be said of its possible significance. The amounts of coproporphyrin from the feces of the six control individuals were in every instance too small to permit of isolation in crystalline form. The amount was smallest from the cases of idiopathic hypochromic anemia, and the secondary anemia with the bleeding ulcer. In the latter case considerable protoporphyrin and a small amount of deuteroporphyrin were demonstrated spectroscopi-

cally, as was to be expected following hemorrhage into the gastro-intestinal tract. The new porphyrin described in Part II was not observed in any instance.

#### COMMENT

Kämmerer (10) has recently pointed out that because many of the common foods contain traces of coproporphyrin, conclusions based upon finding it in the feces would have to be guarded. There is little doubt that exogenous traces of coproporphyrin occur in all feces; nevertheless, that it was in sufficient amount in the feces of two pernicious anemia patients to be readily isolated in crystalline form, while this was not possible in one of the same cases after liver had induced a remission, nor in six other control cases, was considered significant. Furthermore, the fact that only coproporphyrin I was isolated from the pernicious anemia cases is believed to indicate that the coproporphyrin which H. Fischer and Zerweck (9), and Borst and Königsdörffer (29) have noted in the urine and marrow in pernicious anemia is coproporphyrin I, whose formation from a chemical standpoint has no direct relation to the formation or destruction of hemoglobin, but must be regarded as occurring because of independent synthesis, for reasons as yet unknown.

#### SUMMARY

Coproporphyrin I has been isolated from the feces of two typical cases of pernicious anemia during relapse and has been identified by virtue of ester melting point and absorption spectrum. In one of these, studied again after liver had induced a remission, the amount was obviously decreased and too small to isolate. In similar amounts of feces from two normal individuals, and four with anemias of other type, the amounts were too small to permit of isolation.

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# THE RESISTANCE TO FIBRINOLYTIC ACTIVITY OF THE HEMOLYTIC STREPTOCOCCUS WITH SPECIAL REFERENCE TO PATIENTS WITH RHEUMATIC FEVER AND RHEUMATOID (ATROPHIC) ARTHRITIS<sup>1</sup>

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(Received for publication September 11, 1934)

Recently Tillett and Garner (1) have demonstrated that broth cultures of hemolytic streptococci of human origin rapidly liquefy the fibrin-clot of human plasma. Cultures of other species of bacteria obtained from human beings fail to exhibit this property. Tillett, Edwards and Garner (2) further found that the plasma clot from patients convalescent from acute hemolytic streptococcal infections was highly resistant to the action of broth cultures of a human hemolytic streptococcus. The observations of Tillett and his coworkers were confirmed by Hadfield, Magee and Perry (3).

Previously, we (4, 5, 6) have studied groups of patients with rheumatic fever and rheumatoid arthritis to determine what, if any, relationship exists between streptococcal infection and these diseases. In continuing these studies, we have determined the resistance to fibrinolysis of the blood plasma of patients with rheumatic fever and rheumatoid arthritis and of control groups of patients.

## METHODS OF STUDY

All patients were studied while in the hospital. The methods employed in the determination of the resistance to fibrinolysis of blood plasma followed closely the method described by Tillett and his coworkers (1, 2).

At weekly intervals 5 cc. of blood were collected. Potassium oxalate, 0.01 gram per 5 cc. of blood, was employed as an anticoagulant. The plasma was separated by centrifugalization and was used within two hours after its withdrawal.

A strain of the hemolytic streptococcus supplied by Dr. Tillett and designated as CO was employed in all the tests. Freshly grown 18 to 24-

hour cultures of this organism were grown in veal muscle infusion broth, adjusted to a pH of 7.2 and containing one per cent of peptone and 0.2 per cent of  $\text{Na}_2\text{HPO}_4$ .

To 0.2 cc. of fresh oxalated plasma was added 0.8 cc. of sterile physiological salt solution. To this 0.5 cc. of a fresh broth culture of the CO strain of the hemolytic streptococcus was added and well mixed. Then 0.25 cc. of a 0.25 per cent sterile solution of  $\text{CaCl}_2$  was added and well mixed. The tubes were placed immediately in a water bath at  $37.5^\circ \text{C}$ . With repeated observations the times of solid coagulation and, finally, complete dissolution of the clot were recorded. All tests in which the plasma clot was resistant to dissolution after 24 hours' incubation were arbitrarily terminated.

The classification of the degree of resistance of the plasma clot as suggested by Tillett, Edwards and Garner (2) was followed. The highest degree of resistance shown by a patient's plasma during the period of observation was utilized in the classification shown in Table I.

## RESULTS

Determinations of the resistance to fibrinolysis of 520 samples of plasma from 135 individuals were made. Their ages varied between thirteen and seventy years. No correlation could be established between the age of the individual and the ability to develop resistance to fibrinolysis.

The plasmas from groups of individuals without evidence of hemolytic streptococcal infection were studied. Samples of plasma from fourteen apparently normal laboratory workers were followed at frequent intervals for several months. A group of twenty-two patients with pulmonary tuberculosis, lobar pneumonia, herpes zoster and lung abscess were studied on fifty-one occasions.

<sup>1</sup> This investigation was aided in part by a grant from the Proctor Fund of the Harvard Medical School for the Study of Chronic Diseases.



FIG. 1. CRYSTALS OF THE PORPHYRIN ESTER FROM THE FECES IN CASE 1.

$m\mu$ , IV 533.7  $m\mu$  to 523.6, maximum 528.9  $m\mu$ , V 504.8  $m\mu$  to 484.9, maximum 496.1  $m\mu$ . Order of intensity: V, I, IV, III, II. The spectrum was further compared with that of the new porphyrin described in Part II, and found to differ. It is possible that this is the same porphyrin as that obtained repeatedly by H. Fischer and Duesberg (5) from the feces of rabbits. The ester melting point of the porphyrin described by them varied between 182° and 187° C. Its absorption spectrum was evidently more nearly that of a deuteroporphyrin than in the present instance, and the authors believed it to be either an impure deuteroporphyrin III, or an isomer. The same may be true of the porphyrin described above. In the absence of positive identification, nothing can be said of its possible significance. The amounts of coproporphyrin from the feces of the six control individuals were in every instance too small to permit of isolation in crystalline form. The amount was smallest from the cases of idiopathic hypochromic anemia, and the secondary anemia with the bleeding ulcer. In the latter case considerable protoporphyrin and a small amount of deuteroporphyrin were demonstrated spectroscopi-

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## METHODS OF STUDY

All patients were studied while in the hospital. The methods employed in the determination of the resistance to fibrinolysis of blood plasma followed closely the method described by Tillett and his coworkers (1, 2).

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## RESULTS

Determinations of the resistance to fibrinolysis of 520 samples of plasma from 135 individuals were made. Their ages varied between thirteen and seventy years. No correlation could be established between the age of the individual and the ability to develop resistance to fibrinolysis.

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<sup>1</sup> This investigation was aided in part by a grant from the Proctor Fund of the Harvard Medical School for the Study of Chronic Diseases.

TABLE I  
Results of fibrinolytic tests with the plasma clot

	Num- ber of patients	Num- ber of tests	Dissolution time			Maximum resistance (no dis- solution in 24 hours). Number of patients	Marked resistance (dissolu- tion in 8 to 24 hours). Number of patients	Definite resistance (dissolu- tion in 1 to 8 hours). Number of patients	Suscepti- bility (dissolu- tion in 30 to 60 minutes). Number of patients	High sus- ceptibility (dissolu- tion before 30 minutes). Number of patients
			Mini- mum	Maxi- mum	Aver- age					
Normal individuals.....	14	69	14'	5° 21'	1° 2'	0	0	8	4	2
Infections not caused by hemolytic streptococci.	22	51	19'	24°	4° 30'	2	0	13	6	1
Gonococcal arthritis.....	6	30	12'	24°	5° 47'	2	0	3	1	0
Erysipelas.....	25	72	18'	24°	18° 15'	22	0	2	1	0
Other acute hemolytic streptococcal infections	16	39	50'	24°	16° 17'	11	2	3	0	0
Acute glomerular nephri- tis.....	4	13	1° 23'	12°	6° 24'	0	2	2	0	0
Subacute bacterial endo- carditis.....	3	8	13'	24°	11° 38'	2*	0	0	0	1†
Rheumatic fever.....	34	195	7'	24°	19° 14'	29	2	2	0	1
Rheumatoid (atrophic) arthritis.....	11	43	14'	24°	6° 11'	2	0	5	2	2
Total.....	135	520								

\* Associated with *Streptococcus viridans*.

† Associated with an indifferent streptococcus.

In addition, thirty samples of plasma from six patients with gonococcal arthritis were tested. The results of the tests of the plasma of these forty-two individuals are summarized in Table I.

From time to time relatively small variations were noted in the time of dissolution of the plasma clot of normal individuals. Such fluctuations were probably due to variations in the concentration of the fibrinolysin in the cultures. The employment of samples of plasma from the same individual repeatedly in each set of tests served as a satisfactory control. It was unusual to obtain a result which showed more than "definite resistance" to fibrinolysis in the control group. In but four, or 9.5 per cent, of the whole group of individuals without evidence of infection with the hemolytic streptococcus did any samples of plasma show great resistance to fibrinolysis.

For comparison with normal individuals and those with rheumatic fever and rheumatoid arthritis, the effect of acute infection with the hemolytic streptococcus on the resistance to fibrinolysis was studied. In Table I is a summary of the results of seventy-two tests done on the plasma from sixteen patients with other acute hemolytic streptococcal infections such as acute tonsillitis and acute otitis media.

"Maximum resistance" and "marked resist-

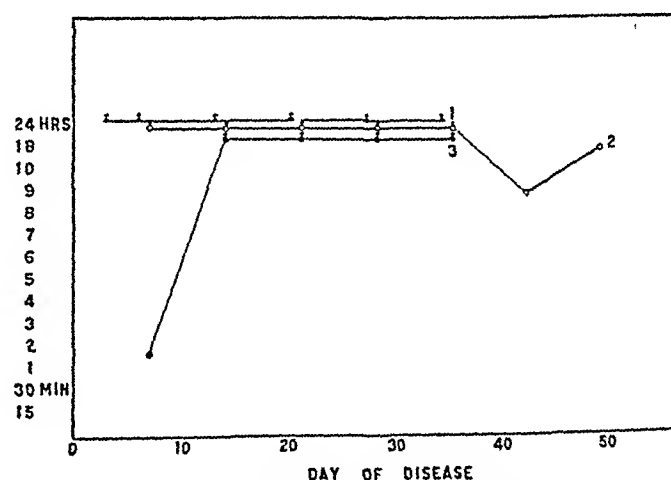


FIG. 1. THE CHANGES IN THE DISSOLUTION TIME OF THE PLASMA CLOT OF THREE PATIENTS WITH ERYSIPELAS.

ance" was encountered in thirty-three, or 80.5 per cent, of these forty-one patients. Changes in the resistance to fibrinolysis were observed in many of these patients. Such changes are shown in Figure 1. Ten patients with erysipelas were tested whose plasma clot repeatedly remained resistant to fibrinolysis after twenty-four hours. In nine patients the resistance was observed to increase during the early period of the illness, later to remain undissolved after twenty-four hours. Three additional patients with erysipelas showed moderate or slight decrease in the resist-

ance of their plasma clot to dissolution. Similar observations were noted in patients with other hemolytic streptococcal infections. Figure 2 shows the results of repeated studies of the resistance of the plasma of a patient with an acute tonsillitis. It is of interest to note that a few patients with undoubted infection with the hemolytic streptococcus failed to develop an enhanced resistance to fibrinolysis during or subsequent to their illness.

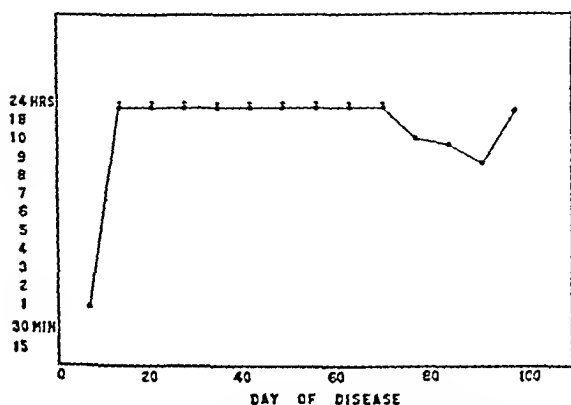


FIG. 2. THE CHANGES IN THE DISSOLUTION TIME OF THE PLASMA CLOT OF A PATIENT WITH ACUTE TONSILLITIS.

Tests were done on four patients with acute glomerular nephritis associated with infection with hemolytic streptococci. The plasma of none of these patients had "maximum resistance" to fibrinolysis.

Of the three patients with subacute bacterial endocarditis the plasma of two, from whose blood a streptococcus viridans was cultured, showed "maximum resistance" to fibrinolysis. From the third patient an "indifferent" streptococcus was obtained; the plasma of this patient was repeatedly highly susceptible to fibrinolysis.

One hundred and ninety-five samples of plasma from thirty-four patients with undoubted active rheumatic fever were tested. The results of these tests are summarized in Table I. Seventeen of the thirty-four patients gave adequate evidence for considering their illnesses as exacerbations of the rheumatic process. Twenty-eight of the thirty-four patients were studied at weekly intervals for four weeks or longer.

In twenty-nine of these patients, or 85.3 per cent, "maximum resistance" was encountered.

In Figure 3 are illustrated the results obtained in three illustrative patients. Of these twenty-nine patients, twenty were studied for periods varying between five and fifteen weeks. The samples of plasma from thirteen of the twenty patients were resistant to fibrinolysis for twenty-four hours persistently; Curve Number 1 in Figure 3 is an example of this group. Five additional patients showed variation in the resistance of their plasma as is illustrated by Curve No. 2 in Figure 3. In each of these the plasma resisted fibrinolysis for twenty-four hours when the patient was first seen and later dissolution was observed within the twenty-four hour period. In but one patient was the earlier sample of plasma dissolved after two and one-half hours; at all subsequent tests it remained unchanged after twenty-four hours, as had been observed in the acute hemolytic streptococcal infections.

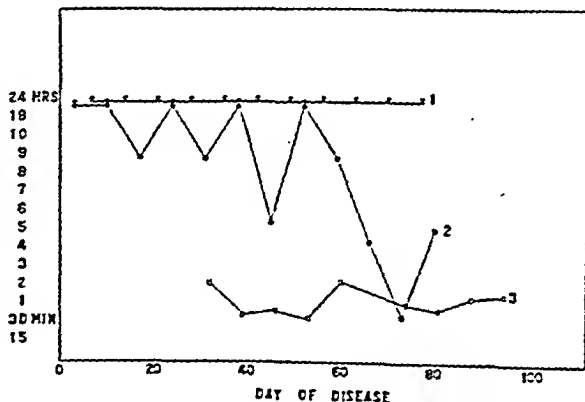


FIG. 3. THE CHANGES IN THE DISSOLUTION TIME OF THE PLASMA CLOT OF THREE PATIENTS WITH RHEUMATIC FEVER.

As in patients with hemolytic streptococcal infections a few patients with rheumatic fever failed to show an enhanced resistance to fibrinolysis, even when followed for several weeks. The results of the tests of the plasma of one such patient are given in Figure 3.

Evidence was obtained in twenty-nine of the thirty-four patients of infection with the hemolytic streptococcus, either from a history of such an infection from one to eight weeks previous to, or as a result of, bacteriological studies at the time of the first anti-fibrinolytic test. In two patients whose plasma showed "maximum resistance" and in one each of those who are classified as



showing "marked resistance," "definite resistance" and "high susceptibility," respectively, evidence of infection by the hemolytic streptococcus was lacking; there was no history or signs of such an infection and throat cultures did not reveal hemolytic streptococci.

In the eleven patients with active joint disease due to rheumatoid arthritis, forty-three samples of plasma were tested. The results are summarized in Table I. Of the two patients, samples of whose plasma were resistant to fibrinolysis for twenty-four hours, one was followed during an attack of acute sinusitis. Before the onset of the sinus infection the fibrin-clot was dissolved in two hours; the fibrin-clot became and remained resistant for twenty-four hours during the six weeks of observation. The observation of the antifibrinolytic properties of the plasma of these eleven patients was similar to the results obtained in the groups of individuals without infection with the hemolytic streptococcus.

#### COMMENTS

From the data presented, certain deductions are justifiable. It is evident that the blood plasma of normal individuals and patients without evidence of hemolytic streptococcal infection may contain antifibrinolysin in varying amounts. In a few individuals with infections due to microorganisms other than the hemolytic streptococcus, the resistance of the blood plasma to fibrinolysis may be high. In view of the observations that enhanced resistance to fibrinolysis may persist for an indeterminate time following a hemolytic streptococcal infection, it is possible that the patients with a high resistance may have had a previous hemolytic streptococcal infection some weeks before examination, in spite of the negative history. In the main, these results are in agreement with the observations of Tillett, Edwards and Garner (2) and Hadfield, Magee and Perry (3).

The blood plasma from patients with recent hemolytic streptococcal infections is generally much more resistant to fibrinolysis than that from control groups of patients. The variations observed are probably due to inherent differences in the reaction of the individual or to the variation in the ability of the infecting organism to call forth antifibrinolysins in man. That there are

differences in the various strains of hemolytic streptococcus, depending upon their source and cultural characteristics, has been emphasized by Tillett (7), De Venter and Reich (8) and Hadfield, Magee and Perry (3). Inasmuch as different strains of hemolytic streptococci vary in their capacity to produce substances such as hemolysin and toxin, it is not surprising that they show differences in their fibrinolysin production.

We believe that the antifibrinolysis is an immune response to infection by hemolytic streptococci which are capable of producing fibrinolysin. The ability of these organisms to produce fibrinolysis may explain in part why the hemolytic streptococcus is so invasive in character, and it most certainly explains the character of the exudate that is seen early in the course of hemolytic streptococcal infection.

In view of the frequency with which rheumatic fever was preceded by infection with the hemolytic streptococcus in these cases, it was not surprising that the plasma from patients with rheumatic fever showed an enhanced resistance to fibrinolysis. The results in this group of patients with rheumatic fever here studied are in close agreement with those observed in patients with acute hemolytic streptococcal infection.

The plasma of patients with active rheumatoid arthritis gave results in all respects similar to that observed in patients without hemolytic streptococcal infection. In but one patient was there evidence of active infection by the hemolytic streptococcus. It would seem that the disease process in the majority of these patients was not associated or preceded by an active hemolytic streptococcal infection.

#### SUMMARY AND CONCLUSIONS

1. The plasma of patients with proven hemolytic streptococcal infection is more highly resistant to fibrinolysis than plasma from normal individuals and patients with infection caused by other microorganisms.

2. The resistance to fibrinolysis of the plasma of patients with rheumatic fever is comparable to that observed in patients with erysipelas and other acute hemolytic streptococcal infections.

3. Rheumatoid (atrophic) arthritis is not ac-

accompanied by an increase in the antifibrinolytic property of the plasma.

We acknowledge our thanks to Miss Marjorie Jewell and Miss Eleanor Fleming for technical assistance.

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# ANTI-TRYPTIC ACTIVITY OF SYNOVIAL FLUID IN PATIENTS WITH VARIOUS TYPES OF ARTHRITIS<sup>1</sup>

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For many years it has been known that blood plasma is capable of inhibiting the action of proteolytic ferments, and especially trypsin. The presence of this property of blood plasma probably plays a part in limiting the damage to tissue by inflammatory exudates containing large numbers of polymorphonuclear leukocytes. In view of the fact that blood plasma possesses anti-tryptic properties, it was of interest to us to determine whether or not similar properties could be detected in synovial fluid from patients with various types of arthritis. Further, we were interested in knowing whether these qualities of the synovial fluid fluctuated in various cases. In all, we studied sixty-five samples of synovial fluid from forty-five cases of arthritis, including thirty cases of gonococcal arthritis, five cases of rheumatic fever, six cases of rheumatoid arthritis and four cases in which there was an accumulation of synovial fluid into a joint following injury. Inasmuch as the procedures differed with various experiments, the methods will be described with the individual experiments.

## *The demonstration of anti-tryptic substances in synovial fluid*

### *Presentation of data:*

The first set of experiments was designed to determine whether or not synovial fluid would inhibit the action of trypsin when it was mixed with casein. One cubic centimeter of 1 per cent trypsin solution was mixed with 5 cc. of 1 per cent solution of casein, made up to a volume of 10 cc. and placed in the incubator at 37° C. for twenty-four hours. At the same time, similar mixtures containing synovial fluid, 0.1, 0.2, 0.4 and 0.8 cc., respectively, were incubated for twenty-four hours. At the end of this period, nonprotein

nitrogen determinations were made on all of the mixtures to determine the amount of protein digested. Synovial fluid alone was incubated for twenty-four hours and the nonprotein nitrogen content of this fluid together with that of the trypsin-casein mixture without synovial fluid served as controls. In all, thirty-two synovial fluids were examined in this way. In sixteen the inhibiting power of 1 cc. of synovial fluid was determined whereas in the remaining sixteen varying amounts of synovial fluid, from 0.1 to 0.8 cc., were studied. A typical example of the experiment is shown in Table I, and the results obtained in the thirty-two cases are charted in

TABLE I

*Example of experiment showing the effect of adding varying quantities of synovial fluid to a mixture of trypsin and casein.*

Tube number.....	1	2	3	3	4	5	7	8
Trypsin 1 per cent solution, cc.....	1	0	1	1	1	1	1	0
Casein 1 per cent solution, cc.....	5	0	5	5	5	5	0	5
Synovial fluid, cc...	0	2	0.1	0.2	0.4	0.8	0	0
Saline solution, cc...	4	8	3.9	3.8	3.7	3.6	9	4
Nonprotein nitrogen, mgm. per 100 cc...	50	8.1	65	65	82	92	15	0

Figure 1. From this figure one may observe that some samples of synovial fluid were capable of inhibiting the action of trypsin on casein solution and others were not. It is seen in individual cases that when synovial fluid inhibited the digestion of casein by trypsin, the larger the amount of synovial fluid used the greater the inhibition. This was probably due to the addition of larger amounts of anti-tryptic substances with increasing amounts of synovial fluid. When there was little or no inhibiting effect present, the amount of digestion increased when large amounts of synovial fluid were added to the mixture. This was prob-

<sup>1</sup> This investigation was aided in part by a grant from the Proctor Fund of the Harvard Medical School for the Study of Chronic Diseases.

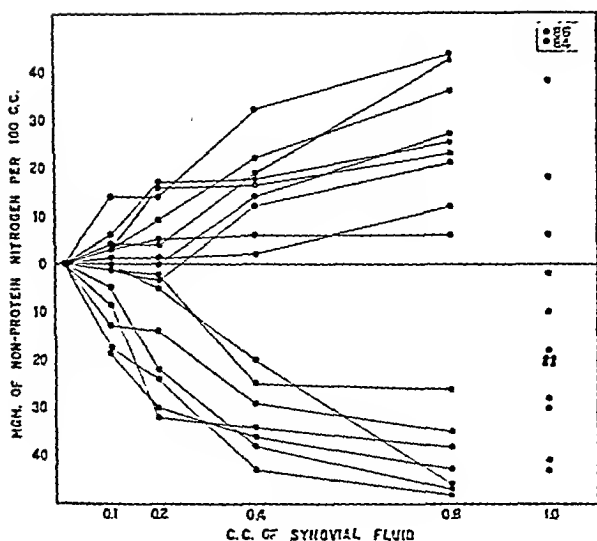


FIG. 1. THE DIFFERENCE IN NONPROTEIN NITROGEN LIBERATED AFTER ADDING VARYING AMOUNTS OF SEVERAL SYNOVIAL FLUIDS TO CONTROL TRYPSIN-CASEIN MIXTURES.

The zero line represents the nonprotein nitrogen found in the control trypsin-casein mixtures. Each line represents a particular synovial fluid. The dots at the extreme right of the chart indicate the difference in nonprotein nitrogen liberated above or below that of control trypsin-casein mixtures, where 1 cc. of each of sixteen synovial fluids was added.

ably due in part to the increased amount of protein substrate contained in the synovial fluid.

From these observations, one may conclude that in some samples of synovial fluid there are substances which are capable of inhibiting the action of tryptic ferments when added to a mixture of trypsin and casein; in others they can not be demonstrated. In order to determine whether or not these properties could be removed by the addition of chloroform to synovial fluid, as is the case with blood plasma (1), ten samples of synovial fluid were studied before and after they had been mixed with chloroform. One cubic centimeter of synovial fluid was mixed with 1 cc. of 1 per cent solution of trypsin and 5 cc. of 1 per cent casein solution, made up to a volume of 10 cc. with normal salt solution and incubated for twenty-four hours at 37° C. At the same time, a similar amount of synovial fluid, which had been mixed with equal amounts of chloroform and incubated for two hours at 37° C. and then separated, was studied in like manner. The trypsin-casein mixture without synovial fluid was

incubated as a control. In addition, the nonprotein nitrogen of two samples of synovial fluid alone was determined after incubation for twenty-four hours. One sample was mixed with chloroform and the other was not. A summary of these experiments appears in Figure 2. It is plain that when chloroform was added to synovial fluid anti-tryptic substances were removed inasmuch as the digestion of the protein was always greater with synovial fluid which had been treated with chloroform. A typical experiment is shown in Table II.

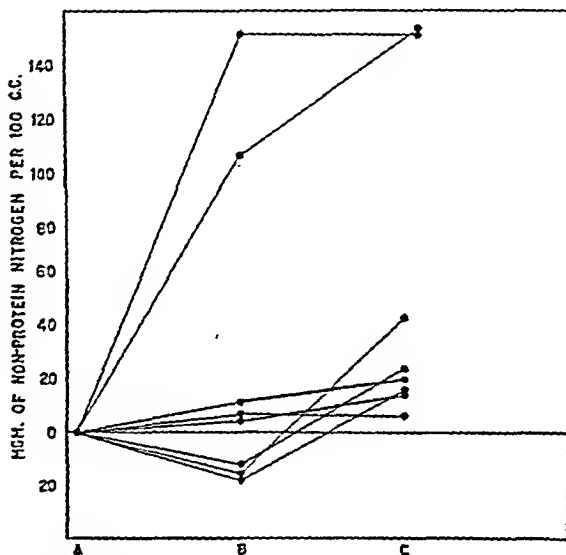


FIG. 2. THE DIFFERENCE IN NONPROTEIN NITROGEN LIBERATED AFTER ADDING TO CONTROL TRYPSIN-CASEIN MIXTURES 1 CC. OF SEVERAL SYNOVIAL FLUIDS (B) OR 1 CC. OF THESE SAME SYNOVIAL FLUIDS AFTER THEY HAD BEEN EXTRACTED WITH CHLOROFORM (C).

The zero line represents the nonprotein nitrogen found in the control trypsin-casein mixtures.

In another series of experiments, the ability of trypsin to digest the protein of the synovial fluid without the addition of casein was studied. In this way it was also possible to determine differences in the anti-tryptic activity of synovial fluid. One cubic centimeter of trypsin was added to 2 cc. of synovial fluid and made up to a volume of 10 cc. with sterile normal salt solution and incubated at 37° C. for twenty-four hours; then nonprotein nitrogen determinations were made to discover how much protein had been digested. The nonprotein nitrogen content of synovial fluid alone and of the trypsin alone served as controls.

TABLE II

*Example of experiment showing the effect of synovial fluid on the digestion of a trypsin-casein mixture before and after removing the anti-tryptic substances from the synovial fluid.*

Tube number.....	Control			Control	
	1	2	3	4	5
Trypsin 1 cc. 1 per cent solution.....	+	+	+	0	0
Casein 5 cc. 1 per cent solution.....	+	+	+	0	0
Synovial fluid, cc.....	0	1	0	2	0
Synovial fluid after chloroform, cc.....	0	0	1	0	2
Normal saline, cc.....	4	3	3	8	8
Nonprotein nitrogen after 24 hours incubation, mgm. per 100 cc..	43.5	29.5	67.5	8.0	8.0

At the same time, synovial fluid which had been treated with chloroform to remove the anti-tryptic substances was studied in a similar fashion. An example of an experiment and the results are shown in Table III and Figure 3.

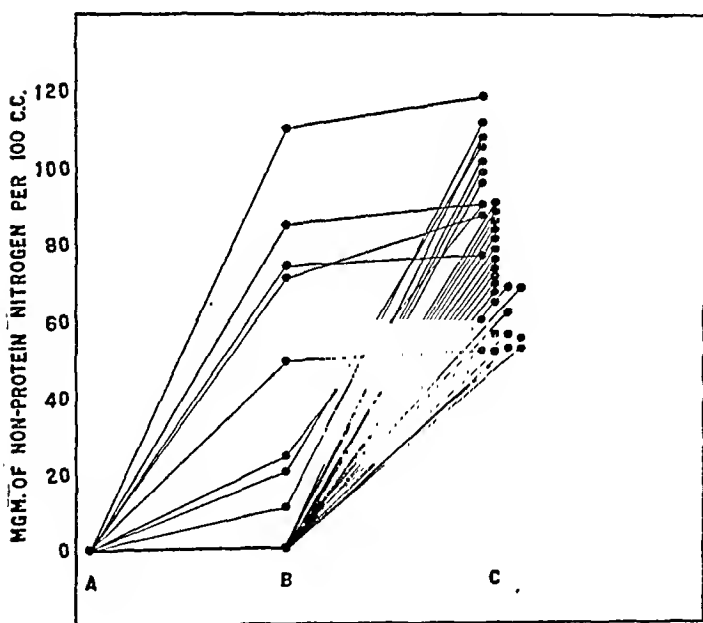


FIG. 3. THE DIGESTION IN MIXTURES OF TRYPSIN WITH SYNOVIAL FLUIDS (B) AND WITH THE SAME SYNOVIAL FLUIDS AFTER THEY HAD BEEN EXTRACTED WITH CHLOROFORM (C).

The zero line represents the sum of the nonprotein nitrogen in the synovial fluid alone and in the trypsin alone.

Twenty-six of the thirty-four samples of synovial fluid completely inhibited the digestive action of 1 cc. of 1 per cent trypsin. In the other eight, some digestion took place indicating that

TABLE III

*Example of experiment to determine the anti-tryptic action of synovial fluid when the protein of the synovial fluid was used as a substrate.*

Tube number.....	1	2	3	4	5
Trypsin, 1 per cent solution, cc....	1	1	0	1	0
Synovial fluid, cc.....	2	0	2	0	0
Normal saline solution, cc.....	7	9	8	7	8
Synovial fluid after chloroform, cc....	0	0	0	2	2
Nonprotein nitrogen, mgm. per 100 cc.....	23	15	8	83	8

the anti-tryptic activity of these fluids was less than the others. When chloroform was added to the fluid to remove the anti-ferment substances, then tryptic digestion was always manifest. The difference in the degree of digestion in the different samples of synovial fluid after they had been treated with chloroform was undoubtedly dependent upon the fact that both the substrate and the free ferment varied in the different samples.

From the above observations, there was no doubt that some samples of synovial fluid contain substances that will inhibit the action of trypsin when it is incubated with casein or the protein of synovial fluid. It was also plain that the anti-tryptic substances could be removed from synovial fluid with chloroform.

We then proceeded to study the various factors that were responsible for the variations in the anti-tryptic activity of the synovial fluid.

#### *Relation of the total number of cells in the synovial fluid and the anti-tryptic content*

Since polymorphonuclear leukocytes contain tryptic ferments and they were present in varying numbers in the different samples of fluid, it was of interest to determine whether there was any correlation between the number of cells in the synovial fluid and its anti-tryptic power. The results of such a study, based on the thirty-two cases recorded in Figure 1, are summarized in Table IV.

From this table, it is seen that, on the whole, the higher the polymorphonuclear cell count the greater the ferment action. In other words, the synovial fluids showing anti-tryptic activity were those with lower cell counts. This was also true of the cases recorded in Figure 2. In six of the

TABLE IV

*Correlation of anti-tryptic potency of synovial fluid and polymorphonuclear cell counts in 32 cases*

Total number of polymorphonuclear cells per c.mm.	Cases showing less digestion than the controls; anti-tryptic activity	Cases showing more digestion than the controls
Less than 10,000 .....	6	7
10,100-20,000 .....	7	2
20,100+ .....	1	9

eight cases in which the fluid showed less anti-tryptic power than the others, the cell count was over 25,000 per c.mm.; whereas of the twenty-six fluids showing higher anti-tryptic power, only six had cell counts above 20,000 per c.mm.

These observations suggested that the diminished anti-tryptic action of some of the fluids was due to the presence of an excess amount of proteolytic ferment derived from the polymorphonuclear leukocytes. Before accepting this conclusion, however, it was necessary to determine three things: (1) the effect of adding increasing amounts of trypsin to constant amounts of protein; (2) the effect of incubating casein with synovial fluids containing varying numbers of leukocytes; and (3) the effect of increasing the amount of protein substrate when the ferment was kept constant.

*Effect of increasing the amount of ferment, keeping the amount of synovial fluid constant*

To determine the effect of increasing the amount of ferment added to a synovial fluid that showed anti-tryptic activity, varying amounts of a 1 per cent solution of trypsin were added to 0.5 cc. of synovial fluid, and the total volume was made up to 10 cc. and incubated at 37° C. for twenty-four hours. As a control the same amounts of ferment were added to synovial fluid after it had been treated with chloroform to remove the anti-tryptic substances. The results of these two experiments were compared with those that were obtained when increasing amounts of trypsin were added to a casein solution. The curves are charted in Figure 4. There was no digestion of the synovial fluid protein with 3 cc. or less of trypsin; however, the digestion was obvious and maximal with 5 cc. (Curve A). When the anti-tryptic substances were removed with chloroform, the digestion was maximal with 3

cc. of trypsin. Moreover, when a substrate such as casein was kept constant and the ferment increased, then digestion increased with increasing amounts of ferment (Curve C). From these experiments it appears that anti-tryptic activity of the synovial fluid may be overcome by an excess of ferment.

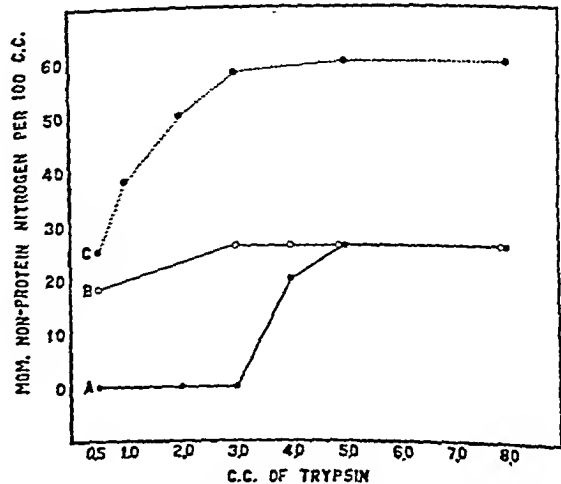


FIG. 4. DIAGRAM ILLUSTRATING THE DIGESTION OF PROTEIN WITH INCREASING AMOUNTS OF FERMENT, ADDED TO A CONSTANT AMOUNT OF SUBSTRATES.

A. Substrate: synovial fluid.

B. Substrate: synovial fluid after the anti-tryptic substances had been removed by chloroform.

C. Substrate: Casein solution.

*Effect of incubating casein with synovial fluids containing varying numbers of leukocytes*

It was necessary, then, to determine the effect of adding samples of synovial fluid containing varying numbers of leukocytes to a solution of casein. Two cubic centimeters of synovial fluid were mixed with 5 cc. of 1 per cent solution of casein and made up to a volume of 10 cc. with normal salt solution. This was incubated at 37° C. for twenty-four hours under conditions insuring sterility. As controls, synovial fluid alone and casein solution alone were incubated at the same time. To be certain that the ferment in the synovial fluid would not be inhibited by the anti-tryptic substances these in a second series were removed with chloroform. In these preparations synovial fluid which had been treated with chloroform was added to the casein solution and studied in the same way. The results of these experiments are summarized in Table V.



TABLE II

*Example of experiment showing the effect of synovial fluid on the digestion of a trypsin-casein mixture before and after removing the anti-tryptic substances from the synovial fluid.*

Tube number.....	Control			Control	Control
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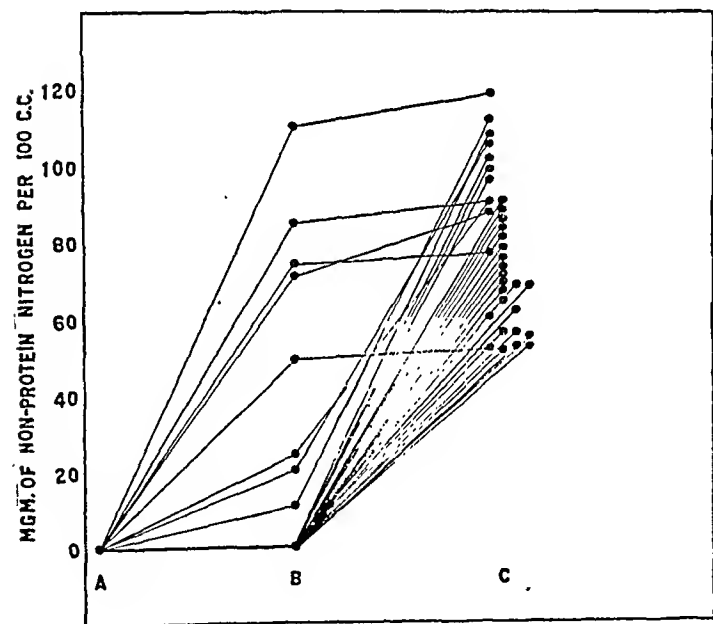


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These observations suggested that the diminished anti-tryptic action of some of the fluids was due to the presence of an excess amount of proteolytic ferment derived from the polymorphonuclear leukocytes. Before accepting this conclusion, however, it was necessary to determine three things: (1) the effect of adding increasing amounts of trypsin to constant amounts of protein; (2) the effect of incubating casein with synovial fluids containing varying numbers of leukocytes; and (3) the effect of increasing the amount of protein substrate when the ferment was kept constant.

*Effect of increasing the amount of ferment, keeping the amount of synovial fluid constant*

To determine the effect of increasing the amount of ferment added to a synovial fluid that showed anti-tryptic activity, varying amounts of a 1 per cent solution of trypsin were added to 0.5 cc. of synovial fluid, and the total volume was made up to 10 cc. and incubated at 37° C. for twenty-four hours. As a control the same amounts of ferment were added to synovial fluid after it had been treated with chloroform to remove the anti-tryptic substances. The results of these two experiments were compared with those that were obtained when increasing amounts of trypsin were added to a casein solution. The curves are charted in Figure 4. There was no digestion of the synovial fluid protein with 3 cc. or less of trypsin; however, the digestion was obvious and maximal with 5 cc. (Curve A). When the anti-tryptic substances were removed with chloroform, the digestion was maximal with 3

cc. of trypsin. Moreover, when a substrate such as casein was kept constant and the ferment increased, then digestion increased with increasing amounts of ferment (Curve C). From these experiments it appears that anti-tryptic activity of the synovial fluid may be overcome by an excess of ferment.

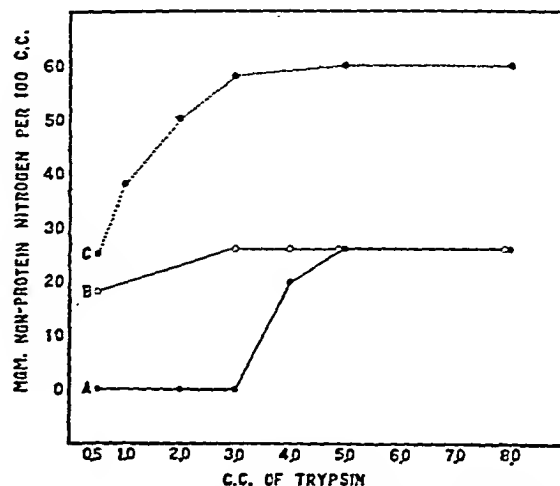


FIG. 4. DIAGRAM ILLUSTRATING THE DIGESTION OF PROTEIN WITH INCREASING AMOUNTS OF FERMENT, ADDED TO A CONSTANT AMOUNT OF SUBSTRATES.

A. Substrate: synovial fluid.

B. Substrate: synovial fluid after the anti-tryptic substances had been removed by chloroform.

C. Substrate: Casein solution.

*Effect of incubating casein with synovial fluids containing varying numbers of leukocytes*

It was necessary, then, to determine the effect of adding samples of synovial fluid containing varying numbers of leukocytes to a solution of casein. Two cubic centimeters of synovial fluid were mixed with 5 cc. of 1 per cent solution of casein and made up to a volume of 10 cc. with normal salt solution. This was incubated at 37° C. for twenty-four hours under conditions insuring sterility. As controls, synovial fluid alone and casein solution alone were incubated at the same time. To be certain that the ferment in the synovial fluid would not be inhibited by the anti-tryptic substances these in a second series were removed with chloroform. In these preparations synovial fluid which had been treated with chloroform was added to the casein solution and studied in the same way. The results of these experiments are summarized in Table V.

TABLE V

*Results of incubating casein and synovial fluid containing varying numbers of cells*

Case	Synovial fluid. Nonprotein nitrogen	Casein solution 1 per cent. Nonprotein nitrogen	Synovial fluid 2 cc. and 1 per cent casein solution. Nonprotein nitrogen	Synovial fluid 2 cc. after treatment with chloroform and 1 per cent casein solution. Nonprotein nitrogen	Total cell count
	<i>mgm. per 100 cc.</i>	<i>mgm. per 100 cc.</i>	<i>mgm. per 100 cc.</i>	<i>mgm. per 100 cc.</i>	<i>per c. mm.</i>
1	7.1	0	23.4	22.2	40,000
2	6.8	0	18.7	15.7	26,000
3	7.8	0	15.0	12.5	20,000
4	7.1	0	21.0	20.0	24,000
5	10.0	0	32.9	29.4	22,000
6	6.8	0	9.1	8.8	10,000
7	10.0	0	13.4	13.0	2,500
8	7.7	0	8.6	8.6	7,500
9	7.1	0	10.0	10.0	12,000
10	6.0	0	8.3	8.0	12,000
11	6.2	0	6.5	9.0	10,500
12	6.9	0	9.3	9.0	12,000

It is manifest that the samples of synovial fluid placed in the upper part of Table V were capable of digesting casein. In the others, the evidence that the digestion took place was slight or lacking. When the results of digestion were correlated with the total cell count it was found that the cases showing digestion of the casein were those with higher total cell counts, whereas those showing little or no digestion contained fewer cells. It would appear, then, that the presence of large numbers of cells in synovial fluid increases the content of proteolytic ferment and in that way is responsible for increased digestion of the protein. That the lack of digestion of the protein in some samples of synovial fluid was not due entirely to the presence of excess amounts of anti-tryptic substances is borne out by the very slight change in digestion after removing them with chloroform.

*Effect of increasing the protein substrate with a constant amount of ferment*

In the experiment in which increasing amounts of synovial fluid were added to trypsin and casein, the protein substrate was obviously increased (Figure 1). It was necessary, therefore, to study the effect of adding increasing amounts of synovial fluid to a constant amount of ferment. One cubic centimeter of a 1 per cent solution of trypsin was added to varying amounts of synovial fluid and incubated for twenty-four hours. The same procedure was carried out with increasing amounts of synovial fluid which had been previously incubated with chloroform to remove the

anti-tryptic substances. The results are charted in Figure 5. When the anti-tryptic substances were removed from the synovial fluid with

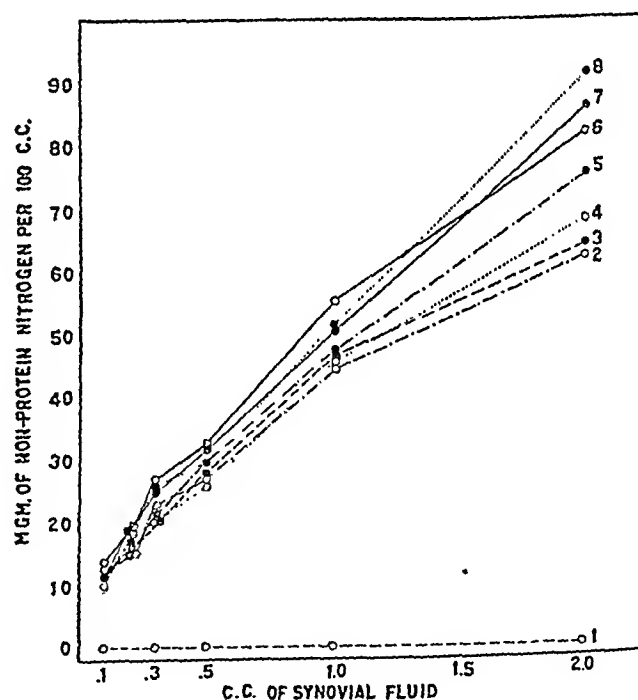


FIG. 5. DIAGRAM ILLUSTRATING THE EFFECT OF DIGESTING INCREASING AMOUNTS OF SYNOVIAL FLUID WHICH HAD BEEN PREVIOUSLY TREATED WITH CHLOROFORM, WHEN THE AMOUNT OF TRYPSIN IS KEPT CONSTANT.

1. Control observations using the seven samples of synovial fluid without preliminary treatment with chloroform.

2 to 8. The amount of digestion above the controls after the seven synovial fluids had been treated with chloroform to remove the anti-tryptic substances.

chloroform there was increased digestion with increasing amounts of synovial fluid. When the anti-tryptic substances were not removed, no digestion of the protein of the synovial fluid was demonstrated. These experiments showed that increasing the amount of synovial fluid was followed by increased digestion of the protein only if the anti-tryptic substances had first been removed.

#### *Comparison of anti-tryptic power of blood plasma and synovial fluid*

In six instances both the blood plasma and synovial fluid from the same patient were studied to compare their anti-tryptic power. The procedure was the same as when the anti-tryptic substances were studied in synovial fluid namely by adding trypsin before and after treatment with chloroform. The results are charted in Figure 6. Both the blood plasma and synovial fluid in 2 cc. amounts were capable of completely inhibiting the action of 1 cc. of 1 per cent trypsin solution. When chloroform was added to the blood plasma or to the synovial fluid the trypsin-inhibiting sub-

stances were removed. The digestive products of the chloroform treated blood plasma protein were higher in each instance than those of the chloroform treated synovial fluid from the same subject. This was attributed to the increased amount of protein substrate available in the blood plasma inasmuch as the protein content of the blood plasma was always higher than that of the synovial fluid from the same individual.

It appears to us that the anti-tryptic substances in these cases at least were probably derived from the exudation of blood plasma into the synovial cavity.

#### DISCUSSION

The foregoing experiments leave little doubt that synovial fluid from some cases of arthritis is capable of inhibiting tryptic digestion. When the anti-tryptic activity was diminished, it was shown that the presence of a large number of leukocytes which contain tryptic ferments was responsible, in part at least, for this reduced anti-tryptic activity. This relation was found regardless of the type of arthritis studied. It is not unreasonable to believe that these anti-tryptic substances are derived from the blood plasma, when due consideration is given to the observations comparing the anti-tryptic activity of blood plasma and synovial fluid from the same individuals.

It would appear, then, that when there is an exudation of fluid into the synovial cavities, the synovial fluid partially protects the tissues from the destruction that might follow the ferment action of destroyed leukocytes. The amount of demonstrable anti-ferment present would depend upon the amount in the blood of the individual but restricted by the amount of free proteolytic enzyme in the synovial fluid. It seems likely that the presence of anti-tryptic substances in synovial fluid is one of the protective mechanisms of the body to prevent destruction in arthritis.

#### SUMMARY AND CONCLUSIONS

1. Synovial fluid contains substances which are capable of inhibiting tryptic digestion.
2. When there are a large number of cells, especially polymorphonuclear leukocytes, present in synovial fluid the anti-tryptic power is usually reduced.

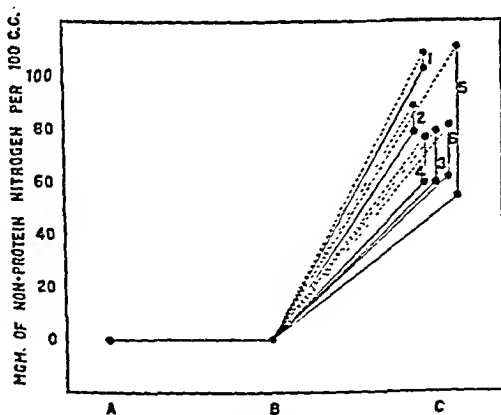


FIG. 6. DIAGRAM COMPARING ANTI-TRYPTIC CONTENT OF BLOOD PLASMA AND SYNOVIAL FLUID FROM SIX PATIENTS.

A. Control for each case. Zero line represents the sum of nonprotein nitrogen in the components of each test measured separately.

B. The amount of digestion after incubation of synovial fluid and trypsin.

C. The amount of digestion of blood plasma and of synovial fluid after removal of anti-tryptic substances from both with chloroform. Solid lines represent synovial fluid. Interrupted lines represent blood plasma.

3. The anti-tryptic substances in synovial fluid can be removed by extraction with chloroform.

4. The anti-tryptic substances in the synovial fluid are probably derived from the blood plasma as both plasma and fluid contain comparable amounts of anti-tryptic substances.

5. One of the properties of synovial fluid is to inhibit the tryptic ferments which are present in exudates.

6. The anti-tryptic power of synovial fluid can be counterbalanced by adding an excess of ferment.

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seems that when the total cell count was low digestion of cartilage did not occur, and that this failure to digest was probably due to a lack of sufficient ferment or to the presence of anti-ferment in the fluid.

#### *Ferment-inhibiting action of synovial fluid*

We next studied the ferment-inhibiting action of synovial fluid when cartilage was used as a substrate and trypsin, instead of leukocytic auto-

In all, fifty-four samples of synovial fluid from forty-five cases of arthritis were studied in this way. They included twenty-three cases of gonococcal arthritis, ten cases of rheumatic fever, seven cases of rheumatoid arthritis, three cases of traumatic arthritis and one each of tuberculous and streptococcal arthritis. The results are summarized in column *A* of Figure 2. It was found that 0.1 cc. of a 1 per cent solution of trypsin was capable of digesting the amount of cartilage used,

TABLE III  
*Example of experiment testing inhibiting action of synovial fluid*

Tube number*	1	2	3	4	5	6	7	8	9	Control					
										10	11	12	13	14	15
Synovial fluid, cc.	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.1	0.1	0	0	0	0	0	0
Buffer solution, cc.	0.8	0.7	0.6	0.5	0.4	0.3	0.2	0.1	0	1.0	0.9	0.8	0.7	0.6	0.5
Trypsin, 1 per cent solution, cc.	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	0	0.1	0.2	0.3	0.4	0.5
Digestion of cartilage.....	0	0	0	0	0	+	+	+	+	0	+	+	+	+	+

\* Each tube contained a piece of cartilage of equal size.

lysate, was employed as the ferment. This was done because the action of trypsin was more prompt than that of leukocytic autolysate.

#### MATERIALS AND METHODS

Small rings of cartilage of approximately equal size were obtained from the trachea of rabbits. These were placed in test tubes with 0.1 cc. of synovial fluid from various patients with arthritis. To these tubes 1 per cent solution of trypsin was added in amounts varying from 0.1 to 0.9 cc. The volume of all the mixtures was made up to 1 cc. with standard buffer solution of pH 8 and placed in the water bath at 56° C. for twenty-four hours. As controls, trypsin in varying amounts was added to the cartilage without the synovial fluid, and cartilage in buffer solution without trypsin was incubated for a similar length of time. The amount of trypsin inhibited by 0.1 cc. of synovial fluid was determined from the tube containing the largest amount of trypsin showing no digestion of the cartilage. An example of an experiment is summarized in Table III. In this case, 0.1 cc. of synovial fluid inhibited the digestive activity of 0.5 cc. of trypsin, whereas in the control 0.1 cc. of trypsin was capable of producing complete digestion of the cartilage.

so that it was obvious that synovial fluid was capable of inhibiting tryptic digestion of cartilage.

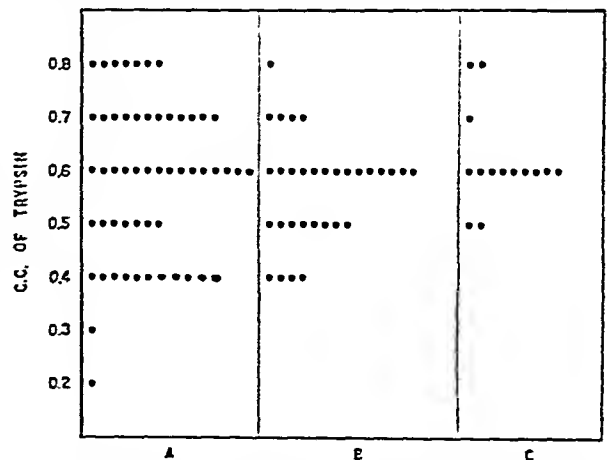


FIG. 2. DIAGRAM ILLUSTRATING THE ANTI-TRYPTIC ACTIVITY OF SYNOVIAL FLUID AND BLOOD PLASMA WHEN CARTILAGE WAS USED AS A SUBSTRATE.

Each dot represents the amount of trypsin inhibited by 0.1 cc. of synovial fluid or blood plasma.

A. Samples of synovial fluid from patients with arthritis.

B. Blood plasma from patients with arthritis.

C. Blood plasma from patients without arthritis.

That the anti-tryptic activity of the synovial fluid was not reduced by bacteria, such as *Staphy-*

TABLE I

*Results of incubating leukocytic autolysate and cartilage at 56° C.*

	pH 4	pH 5	pH 6	pH 7	pH 8	
Leukocytic autolysate, cc. ....	2	2	2	2	2	0
Digestion 4 days. ....	0	0	0	0	0	0
" 6 " .....	0	0	++	++	++++	0
" 10 " .....	++++	++++	++++	++++	++++	0

++ Incomplete digestion  
 ++++ Complete digestion.

*Results of incubating leukocytic autolysate and cartilage at 37° C.*

	pH 4	pH 5	pH 6	pH 7	pH 8	
Leukocytic autolysate, cc. ....	2	2	2	2	2	0
Digestion 4 days. ....	0	0	0	0	0	0
" 6 " .....	0	0	0	0	0	0
" 10 " .....	0	0	0	0	0	0

Figure 1, indicate that some digestion of cartilage by leukocytic autolysate took place at 37° C. though much less than at 56° C.

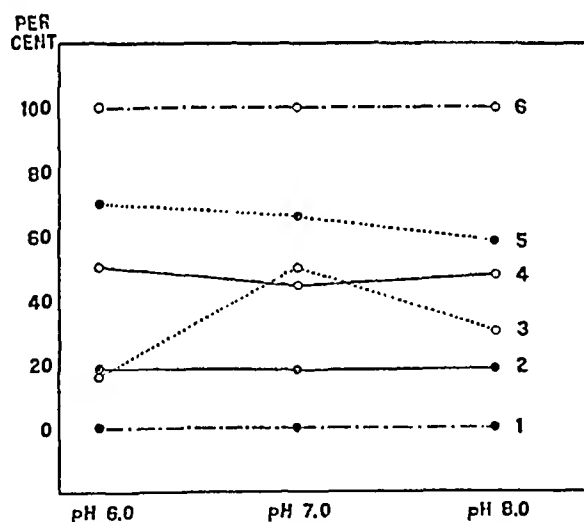


FIG. 1. CHART ILLUSTRATING THE AMOUNT OF DIGESTION OF CARTILAGE BY LEUKOCYTIC AUTOLYSATE, AT pH 6, 7 AND 8 AT 37° C. AND 56° C.

1. Control at 37° C. 2. Leukocytic autolysate from staphylococcus exudate at 37° C. 3. Leukocytic autolysate from pneumococcus exudate at 37° C. 4. Control at 56° C. 5. Same as 2 at 56° C. 6. Same as 3 at 56° C.

### *The effect of synovial fluid on the digestion of cartilage*

Since leukocytic autolysate was found to contain ferments that were capable of digesting cartilage, we determined whether synovial fluid re-

moved from patients with arthritis was capable of digesting cartilage or plated coagulated blood serum. The results of the study of twelve cases are recorded in Table II. Digestion of the cartilage

TABLE II

*Digestion of plated blood serum and of cartilage by synovial fluid*

Case number	Type of arthritis	Cells per c. mm.	Digestion of plated blood serum		Digestion of cartilage	
			37° C.	56° C.	37° C.	56° C.
1	Gonococcal	9,750	0	0	0	0
2	"	7,850	0	0	0	0
3	"	10,000	0	0	0	0
4	"	12,200	0	0	0	0
5	"	12,250	0	0	0	0
6	"	21,000	0	0	0	0
7	"	15,650	0	0	0	0
8	Tuberculous	6,650	0	0	0	0
9	"	11,600	0	0	0	0
10	"	6,500	0	0	0	0
11	Staphylococcal	110,000	+	+	±	++++
12	Staphylococcal	240,000	+	+	±	++++

was not observed in seven samples of synovial fluid from gonococcal arthritis or in three samples from tuberculous arthritis. In the two cases of staphylococcal arthritis, in which there were large numbers of polymorphonuclear leukocytes, digestion was apparent. From these experiments it

seems that when the total cell count was low digestion of cartilage did not occur, and that this failure to digest was probably due to a lack of sufficient ferment or to the presence of anti-ferment in the fluid.

#### *Ferment-inhibiting action of synovial fluid*

We next studied the ferment-inhibiting action of synovial fluid when cartilage was used as a substrate and trypsin, instead of leukocytic auto-

In all, fifty-four samples of synovial fluid from forty-five cases of arthritis were studied in this way. They included twenty-three cases of gonococcal arthritis, ten cases of rheumatic fever, seven cases of rheumatoid arthritis, three cases of traumatic arthritis and one each of tuberculous and streptococcal arthritis. The results are summarized in column *A* of Figure 2. It was found that 0.1 cc. of a 1 per cent solution of trypsin was capable of digesting the amount of cartilage used,

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*Example of experiment testing inhibiting action of synovial fluid*

Tube number* .....	1	2	3	4	5	6	7	8	9	Control					
										10	11	12	13	14	15
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Buffer solution, cc.....	0.8	0.7	0.6	0.5	0.4	0.3	0.2	0.1	0	1.0	0.9	0.8	0.7	0.6	0.5
Trypsin, 1 per cent solution, cc.....	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	0	0.1	0.2	0.3	0.4	0.5
Digestion of cartilage.....	0	0	0	0	0	+	+	+	+	0	+	+	+	+	+

\* Each tube contained a piece of cartilage of equal size.

lysate, was employed as the ferment. This was done because the action of trypsin was more prompt than that of leukocytic autolysate.

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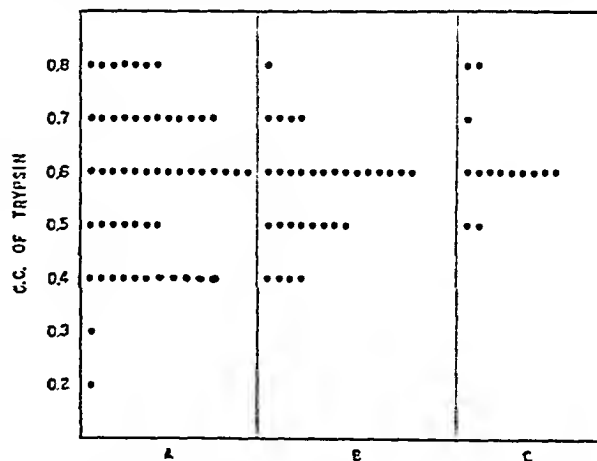


FIG. 2. DIAGRAM ILLUSTRATING THE ANTI-TRYPTIC ACTIVITY OF SYNOVIAL FLUID AND BLOOD PLASMA WHEN CARTILAGE WAS USED AS A SUBSTRATE.

Each dot represents the amount of trypsin inhibited by 0.1 cc. of synovial fluid or blood plasma.

A. Samples of synovial fluid from patients with arthritis.

B. Blood plasma from patients with arthritis.

C. Blood plasma from patients without arthritis.

That the anti-tryptic activity of the synovial fluid was not reduced by bacteria, such as hem-



lytic streptococci, growing in it was shown by the following experiments. Hemolytic streptococci were added in large numbers to twelve samples of synovial fluid and grown at a temperature of 37° C. for twenty-four hours; then the synovial fluid was added to the trypsin and cartilage as in the previous experiments. The results were the same for infected as for non-infected fluids.

TABLE IV

*Comparison of inhibitory action of infected and non-infected synovial fluid*

Number of cases	Amount of trypsin inhibited by 0.1 cc. of synovial fluid	Amount of trypsin inhibited by 0.1 cc. of infected synovial fluid
	cc.	cc.
1	0.5	0.5
8	0.6	0.6
2	0.7	0.7
1	0.8	0.8
12		

*Comparison of anti-tryptic activity of blood plasma and synovial fluid*

It would be surprising if the synovial fluid and the blood plasma did not contain similar amounts of anti-tryptic activity, since synovial fluid is an exudation of blood plasma together with a collection of leukocytes derived from the circulating blood, and cells and mucin derived from the synovial membrane itself. To compare the ability of the blood plasma to inhibit the digestion of cartilage with that of synovial fluid, forty-five samples of blood plasma were studied in the same way as the synovial fluid. The results are charted in Figure 2. The ability of blood plasma to inhibit the digestive action of trypsin when cartilage was used as a substrate was the same as that of synovial fluid, and the variations in the anti-tryptic substances of the blood plasma in patients without arthritis were as great as those with disease of the joints.

*Relation of cell count of synovial fluid to anti-tryptic activity*

In the experiments (1) in which the anti-tryptic activity of synovial fluid was studied, using casein or the protein of the synovial fluid as a substrate, it was found that it was more common to have diminished anti-tryptic activity when the fluid contained large numbers of leukocytes than when they were present in small numbers. Figure 3 was

constructed to demonstrate the lack of correlation between the total cell count and the anti-tryptic activity. At first glance, these observations seem to be in conflict with our previous experiments mentioned above and they require comment. From the experiments summarized in Table II, there could be no doubt that synovial fluid containing large numbers of leukocytes (110,000 and

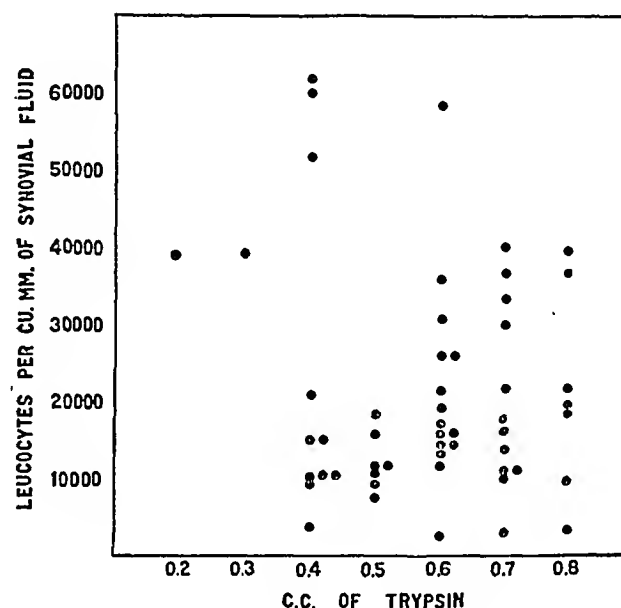


FIG. 3. DIAGRAM SHOWING THE VARIATION OF THE ANTI-TRYPTIC ACTIVITY AND THE TOTAL NUMBER OF LEUKOCYTES IN THE SYNOVIAL FLUID.

Each dot represents the number of leukocytes in 1 c.mm. of synovial fluid and the amount of trypsin the synovial fluid was capable of inhibiting when cartilage was used as a substrate.

240,000) per cubic centimeter was capable of digesting cartilage, whereas those with few leukocytes were not. In the experiments recorded in Figure 3 there were no samples of synovial fluid containing more than 60,000 leukocytes per cubic centimeter and most of them were under 20,000 per cubic centimeter. It seems reasonable to assume, then, that the ferment derived from the leukocytes in the cases studied was not sufficient to enhance the action of the trypsin or decrease the anti-tryptic activity of the synovial fluid which might occur independent of the leukocyte count. It appears, therefore, that cartilage may be protected from the activity of tryptic ferments even in the presence of large numbers of leukocytes. When they become excessive, however, the ferment action predominates and cartilage is destroyed. There was no correlation between the

type of arthritis and the anti-tryptic content of the synovial fluid in our cases.

#### DISCUSSION

From these observations one may conclude that synovial fluid exhibits as much anti-tryptic activity as blood plasma, and that the anti-ferment of synovial fluid is most likely acquired from the blood plasma by exudation of fluid into the joint cavity. Moreover, since anti-tryptic substances were not destroyed by bacteria but were shown to be less active in the presence of an excess of ferment or leukocytic autolysate, it seems reasonable to postulate that when exudates occur in joints the extent of the damage will depend in part upon the excess of ferment over anti-ferment.

Phemister (3) has pointed out that purulent exudates containing polymorphonuclear cells in abundance are capable of digesting small pieces of cartilage and he attributes this to proteolytic ferments from the cells. He showed further that exudates obtained from tuberculous lesions were incapable of digesting cartilage. This he attributed to a lack of proteolytic ferment from cells which he reported were predominately monocytes and lymphocytes. From his observations and from the fact that purulent exudation into joints is often followed by destruction of cartilage, and from the evidence that tuberculous infection is followed by less digestion of cartilage than occurs in other types of arthritis, Phemister concluded that the type of cellular response was important in deciding whether or not extensive destruction of cartilage will take place. Our observations are in complete agreement with his. It is necessary, however, to take into consideration

the presence of anti-tryptic substances as well as the presence of ferments in any given case. We feel that one of the reasons that destruction takes place in one case of acute joint infection and not in all cases of the same kind is that only in some cases are anti-ferment substances not in excess of the ferments. This anti-tryptic activity, when it is present, limits the destruction of tissue; it can, however, be overcome by a great excess of ferment.

#### SUMMARY AND CONCLUSIONS

1. Synovial fluid from patients with arthritis of various types inhibits, in the test tube, the digestion of cartilage.
2. This property is not destroyed by growing hemolytic streptococci in the synovial fluid.
3. Leukocytic autolysates digest cartilage in vitro, especially at 56° C. This action can be inhibited by synovial fluid.
4. The presence of large amounts of anti-tryptic substances in synovial fluid probably prevents or limits the destruction of cartilage by the enzymes liberated in purulent exudates.

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#### *Relation of cell count of synovial fluid to anti-tryptic activity*

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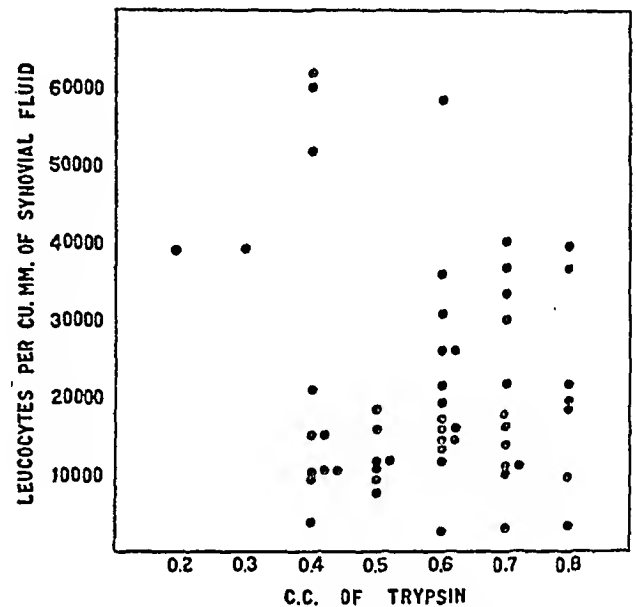


FIG. 3. DIAGRAM SHOWING THE VARIATION OF THE ANTI-TRYPTIC ACTIVITY AND THE TOTAL NUMBER OF LEUKOCYTES IN THE SYNOVIAL FLUID.

Each dot represents the number of leukocytes in 1 c.mm. of synovial fluid and the amount of trypsin the synovial fluid was capable of inhibiting when cartilage was used as a substrate.

240,000) per cubic centimeter was capable of digesting cartilage, whereas those with few leukocytes were not. In the experiments recorded in Figure 3 there were no samples of synovial fluid containing more than 60,000 leukocytes per cubic centimeter and most of them were under 20,000 per cubic centimeter. It seems reasonable to assume, then, that the ferment derived from the leukocytes in the cases studied was not sufficient to enhance the action of the trypsin or decrease the anti-tryptic activity of the synovial fluid which might occur independent of the leukocyte count. It appears, therefore, that cartilage may be protected from the activity of tryptic ferments even in the presence of large numbers of leukocytes. When they become excessive, however, the ferment action predominates and cartilage is destroyed. There was no correlation between the

type of arthritis and the anti-tryptic content of the synovial fluid in our cases.

#### DISCUSSION

From these observations one may conclude that synovial fluid exhibits as much anti-tryptic activity as blood plasma, and that the anti-ferment of synovial fluid is most likely acquired from the blood plasma by exudation of fluid into the joint cavity. Moreover, since anti-tryptic substances were not destroyed by bacteria but were shown to be less active in the presence of an excess of ferment or leukocytic autolysate, it seems reasonable to postulate that when exudates occur in joints the extent of the damage will depend in part upon the excess of ferment over anti-ferment.

Phemister (3) has pointed out that purulent exudates containing polymorphonuclear cells in abundance are capable of digesting small pieces of cartilage and he attributes this to proteolytic ferments from the cells. He showed further that exudates obtained from tuberculous lesions were incapable of digesting cartilage. This he attributed to a lack of proteolytic ferment from cells which he reported were predominately monocytes and lymphocytes. From his observations and from the fact that purulent exudation into joints is often followed by destruction of cartilage, and from the evidence that tuberculous infection is followed by less digestion of cartilage than occurs in other types of arthritis, Phemister concluded that the type of cellular response was important in deciding whether or not extensive destruction of cartilage will take place. Our observations are in complete agreement with his. It is necessary, however, to take into consideration

the presence of anti-tryptic substances as well as the presence of ferments in any given case. We feel that one of the reasons that destruction takes place in one case of acute joint infection and not in all cases of the same kind is that only in some cases are anti-ferment substances not in excess of the ferments. This anti-tryptic activity, when it is present, limits the destruction of tissue; it can, however, be overcome by a great excess of ferment.

#### SUMMARY AND CONCLUSIONS

1. Synovial fluid from patients with arthritis of various types inhibits, in the test tube, the digestion of cartilage.
2. This property is not destroyed by growing hemolytic streptococci in the synovial fluid.
3. Leukocytic autolysates digest cartilage in vitro, especially at 56° C. This action can be inhibited by synovial fluid.
4. The presence of large amounts of anti-tryptic substances in synovial fluid probably prevents or limits the destruction of cartilage by the enzymes liberated in purulent exudates.

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# THE ABILITY OF NEPHRITIC PATIENTS TO DEAMINIZE AND FORM UREA FROM INGESTED GLYCINE

By ESBEN KIRK

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The regular finding in uremic coma of a marked increase in the amino nitrogen concentration of plasma and the frequent occurrence of a moderate elevation during the terminal stage of Bright's disease have recently been reported (Kirk, 1933).

The present study was undertaken to obtain further information about the amino acid metabolism in nephritis, especially during the terminal stage of the disease and in uremia.<sup>1</sup> For this purpose determinations were made of the rate of deamination of ingested amino acids, and of urea formation following the ingestion.

Animal experiments of Van Slyke, Cullen and McLean (1915) and Bollman, Mann and Magath (1924), confirmed by results of Krebs and Henseleit (1932) with the tissue technique of Warburg, have led to the conclusion that the liver is the only important site of urea formation in the body. The experiments of Bollman, Mann and Magath (1926) indicated also that, in the dog at least, the liver is responsible for the deamination of amino acids which precedes urea formation, as amino acids injected into a liverless dog could be recovered quantitatively from the tissues and urine of the animal many hours after the injection. The technique of experiments with dogs does not, however, yield results sufficiently exact to exclude the possibility of a minor amount of deamination in organs other than the liver, because estimation of the amino nitrogen content of the entire animal at the beginning and end of an experiment can be only approximate. By *in vitro* experiments Krebs (1933) found that kidney as well as liver tissue could deaminate amino acids; it therefore appears possible that a minor part of the deamination that occurs *in vivo* may be located in the kidneys.

Several attempts have been made by previous investigators to devise liver function tests based

on observations of blood amino nitrogen or urea curves after ingestion of proteins or amino acids. Such curves might be expected to indicate the efficiency of the deaminizing and urea forming functions, respectively. In general such tests, applied almost exclusively to patients with suspected or obvious liver lesions, have given results of doubtful significance. This may be due to the technique employed. Von Falkenhausen (1924) and Witts (1929) estimated the amino nitrogen concentration of plasma at regular intervals following amino acid ingestion. The amino nitrogen determinations were, however, made by the colorimetric method of Folin (1922), which has later been found to be unsatisfactory for quantitative analysis of blood samples of increased amino nitrogen content (Van Slyke and Kirk, 1933).

A series of investigators (Witts, (1929), Cohen and Levin (1927), Theis (1928)) have attempted to detect abnormal urea formation by studying the blood urea curve following ingestion of proteins or amino acids. However, estimation of the urea excretion in the urine was omitted, and it does not appear that the blood urea curves alone could yield accurate estimates of the rate of urea formation. That normal persons after a protein meal sometimes show no increase in blood urea, but even a decrease, due to the fact that a diuresis may set in and wash out the urea from the body more rapidly than it is formed, was observed by Peters and Van Slyke (1931). Urea formation from ingested protein or amino acids can be quantitatively determined only by estimation of both the amount of urea retained in the body and the amount excreted in the urine.

This is well illustrated by the following figures obtained in a normal person and in a nephritic patient with 30 per cent of kidney function. The urea formation was calculated in a 6-hour period after ingestion of 60 grams of gelatine by the method described in this paper. Although the total urea formation is practically the same in the two individuals the values for retained and excreted urea differ greatly.

<sup>1</sup> The nomenclature for the different types and stages of Bright's disease used in this paper is the same as employed in previous publications from this Hospital (Van Slyke, Stillman, Möller, *et al.*, (1930); Kirk, (1933)).

	Normal individual	Nephritic patient with 30 per cent of kidney function
	mgm.	mgm.
Urea nitrogen excreted in the urine .....	2758	1472
Urea nitrogen retained in the body .....	1433	2774
Total urea nitrogen formation .....	4191	4246

#### PROCEDURE FOR ESTIMATING RATES OF DEAMINIZATION AND UREA FORMATION

For study of the rate of deamination and calculation of the urea formation from ingested amino acids the following test was applied to 5 normal persons, to 3 uremic patients and to 4 patients with marked nephrotic symptoms accompanying the chronic active stage of hemorrhagic Bright's disease.

Twenty-five grams of glycine, dissolved in 500 cc. of water, were given by mouth at 9 a.m. to the subject, who had had nothing to eat since supper on the previous evening. Blood samples were drawn immediately before the glycine ingestion, and at 10 a.m., 11 a.m., 12 noon, 1 p.m., and 3 p.m. The plasma amino nitrogen concentration of all the samples was determined. The specimens drawn at 9 a.m., 12 noon and 3 p.m., further served for urea nitrogen analyses of whole blood. The urine was collected in two 3-hour periods (9 a.m. to 12 noon, and 12 noon to 3 p.m.), the volumes were measured, and the urea nitrogen, ammonia nitrogen and amino nitrogen concentrations were determined. From the urea analyses of blood and urine the urea formation was calculated as the sum of the urea excreted in the urine and the increase of the urea content of the body. The latter was estimated from the blood urea increase, because urea is so rapidly diffusible that its concentration in all the tissues of the body per kilo water present remains approximately equal to that of the blood (Marshall and Davis (1914), Bollman, Mann and Magath (1924)). Hence approximately: Total body urea increase = increase per liter blood  $\times$  body weight in kilos  $\times$  0.8. The factor 0.8 represents approximately the ratio of water content per kilo of whole body to water content per liter of blood.

From the total urea nitrogen values obtained must be subtracted the endogenous urea nitrogen

formation. This is determined on a subsequent day under similar conditions, but without ingestion of glycine. In all calculations of urea formation the urine ammonia nitrogen values were added to the urea nitrogen values.

#### ANALYTICAL METHODS

*Plasma amino nitrogen:* Manometric method of Van Slyke (1929) after destruction of the urea with urease. *Urine amino nitrogen:* Northrop's (1926) formaldehyde titration method as applied to urine by Van Slyke and Kirk (1933). *Blood urea nitrogen:* Gasometric urease method, procedure B, of Van Slyke (1927). *Urine urea nitrogen:* Gasometric urease method of Van Slyke (1927) for urine analyses. *Urine ammonia nitrogen:* Aeration and titration method of Van Slyke and Cullen (1916).

#### RESULTS

The *plasma amino nitrogen curves* obtained in 4 normal persons and in 3 uremic patients are presented in Figure 1. The hatched area in Figure 1 indicates the normal range of plasma amino nitrogen following ingestion of 25 grams of glycine. The individual normal amino nitrogen curves after absorption of the glycine showed great similarity, the maximum value being usually observed one hour after the ingestion. In the uremic patients, however, a much greater rise occurred,<sup>2</sup> even in instances where the fasting amino nitrogen values were normal, probably indicating a retardation of the deamination rate of the absorbed amino acids. Frequently the amino nitrogen values remained considerably elevated for several hours in the blood of the nephritic patients.

The degree of this metabolic dysfunction is illustrated by the following figures: The *increase* in plasma amino nitrogen in the normal individuals after ingestion of 25 grams of glycine was 5 to 7 mgm. per 100 cc., and averaged 5.8 mgm. The highest concentration attained was 13.9 mgm. In the uremic patients, on the other hand, the rise was 2 to 4 times as great as in the

<sup>2</sup> In one uremic patient, a child of 11 years, no change in the amino nitrogen concentration of plasma and no measurable urea formation occurred after ingestion of 20 grams of glycine. It may be supposed that in this case the glycine was not absorbed from the intestine.

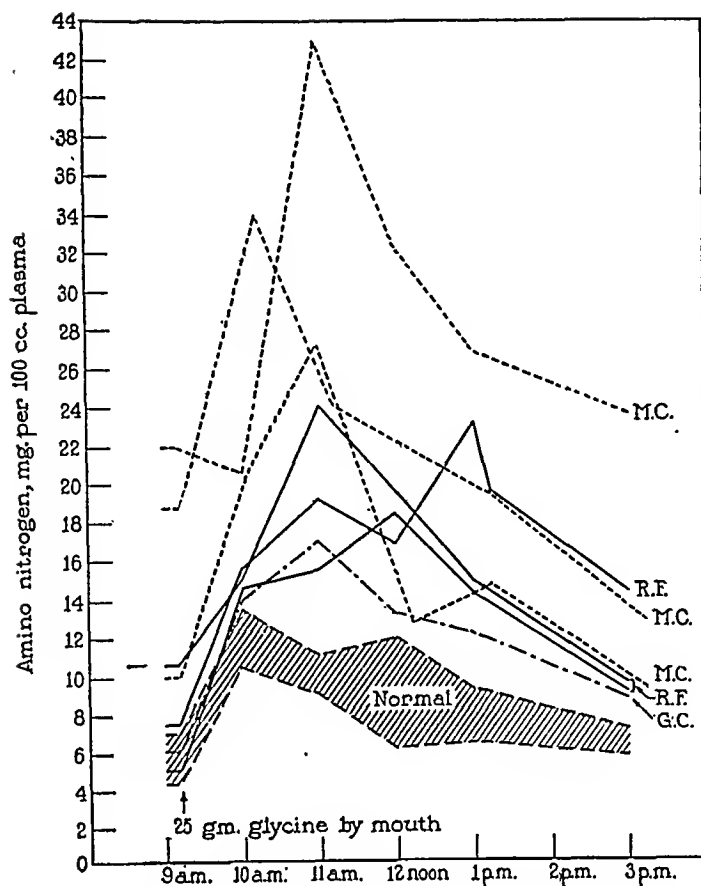


FIG. 1. PLASMA AMINO NITROGEN CURVES AFTER INGESTION OF 25 GRAMS OF GLYCINE.

Four normal subjects and three uremic patients. There are 3 curves each for patients M. C. and R. F., and one curve for G. C.

normal persons, and the plasma amino nitrogen reached values as high as 43 mgm. per 100 cc. The cause of this marked increase does not appear to be impaired excretion of the amino acids in the urine, which, under the conditions used, is only a minor factor in removing amino acids from the circulation. Even in the normal individuals the total excretion of amino nitrogen in the 6-hour period of the test constituted less than 5 per cent of the ingested amount. It is possible that the tissues of uremic patients are more nearly saturated with amino acids than the tissues of normal individuals, and that for this reason the glycine passes less rapidly and completely from blood to tissues in the uremic subject. Such pre-saturation of the tissues would not occur, however, if the deaminizing function were previously normal. Therefore, even if the direct cause of the high and prolonged blood amino nitrogen curve were previous saturation of the tissues with amino acids, the primary cause would be retarded deaminization.

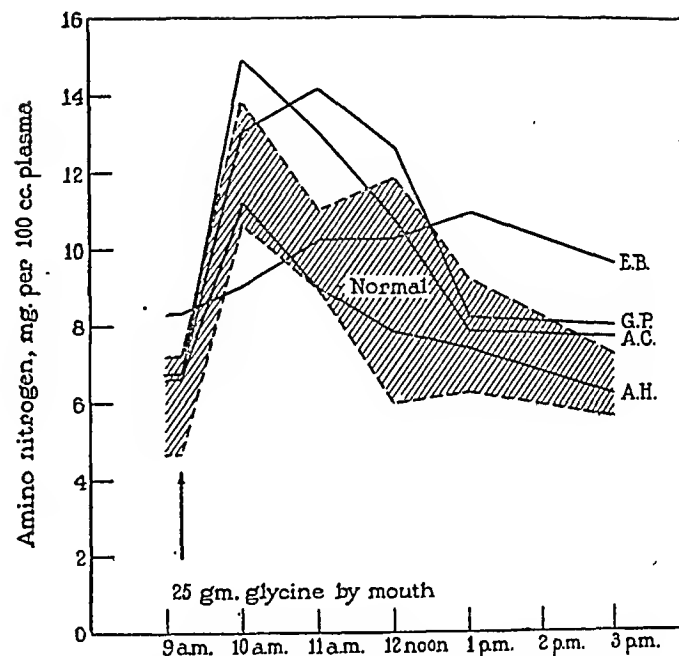


FIG. 2. PLASMA AMINO NITROGEN CURVES AFTER INGESTION OF 25 GRAMS OF GLYCINE.

Four patients in the chronic active stage of hemorrhagic nephritis, with the nephrosis syndrome predominating.

The plasma amino nitrogen curves obtained in 4 patients with marked nephrotic syndrome accompanying the chronic active stage of hemorrhagic Bright's disease are illustrated in Figure 2. These patients still had urea clearances above 20 per cent of normal, and hence would not be classed as terminal (Van Slyke, Stillman, *et al.*, 1930). The postabsorptive plasma amino nitrogen curves fall nearly within the area which includes the curves obtained in the normal individuals.

The rates of urea nitrogen formation, following the ingestion of 25 grams of glycine by the same normal and nephritic subjects, are presented in Figure 3.

Whereas the normal persons in the first 3 hours following the glycine feeding transformed an average of 48 per cent of the ingested 4670 mgm. of amino nitrogen into urea nitrogen, a much smaller urea formation (9.8 and 24 per cent) was found in two of the three uremic patients in the terminal stage.

On the other hand, the patients in the chronic active stage, with symptoms chiefly nephrotic, formed urea at the same rate as normal subjects.

It is evident that the processes by which absorbed amino acids are removed from the blood stream and transformed into urea proceed at a

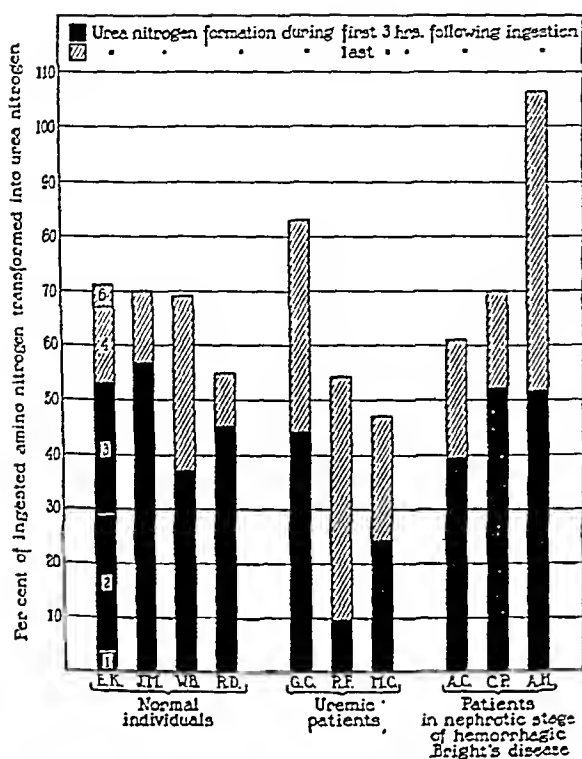


FIG. 3. UREA FORMATION FROM 25 GRAMS OF INGESTED GLYCINE.

In the first column the numbers indicate successive hours after the ingestion.

normal rate until the terminal stage of nephritis is reached: then retardation of these processes frequently occurs.

#### *Urea synthesis from ammonia*

The retarded urea formation observed in the terminal stage of nephritis might be a consequence of delay in either deamination of the ingested glycine, or in the synthesis of urea from the nitrogen split off by deamination. Considerable urea formation during the second 3-hour period in Case R. F. presented in Figure 3 would suggest that the retarded initial formation of urea was dependent on delayed deamination.

In order to obtain more direct data on the efficiency of urea synthesis in uremia, experiments were undertaken in which material for the urea synthesis was supplied directly as ammonia nitrogen instead of in the form of amino acid nitrogen. The investigation included a study of the blood ammonia curve in normal and uremic individuals following ingestion of ammonium citrate, and cal-

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At 9 a.m. 13.3 grams of ammonium citrate (containing 2950 mgm. of ammonia nitrogen) were given dissolved in water to the patient, who had been fasting since supper on the previous evening.<sup>4</sup> Blood samples for ammonia determination were drawn immediately before the ingestion and at 9:30 a.m., 10 a.m., and 12 noon. The ammonia analyses were made by the method of Van Slyke and Hiller (1933), the analyses being started less than 3 minutes after the blood samples were drawn. Calculation of the urea formation in the 3-hour period was performed as described above under the glycine test.

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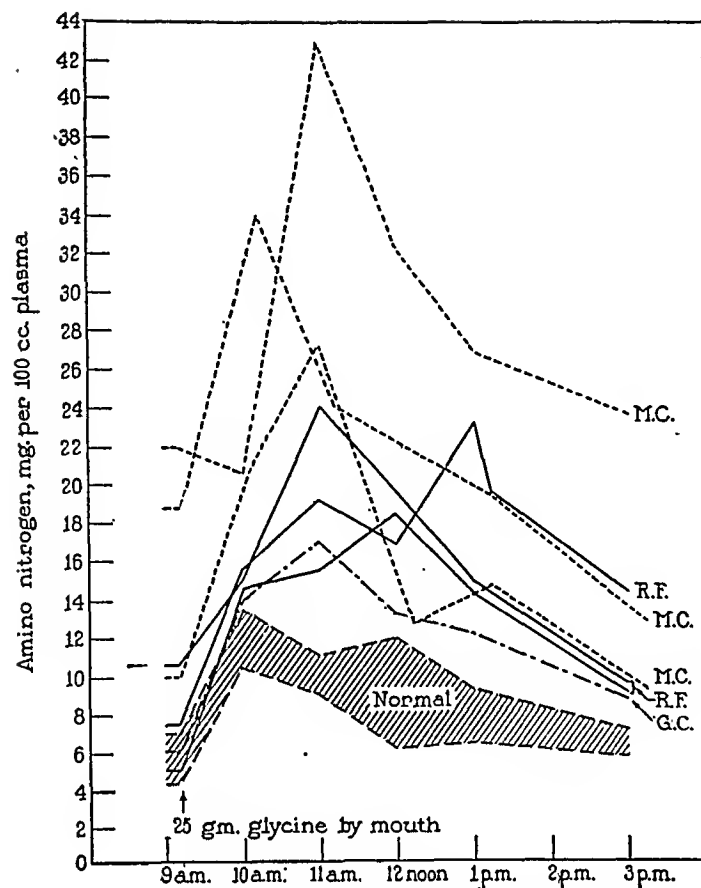


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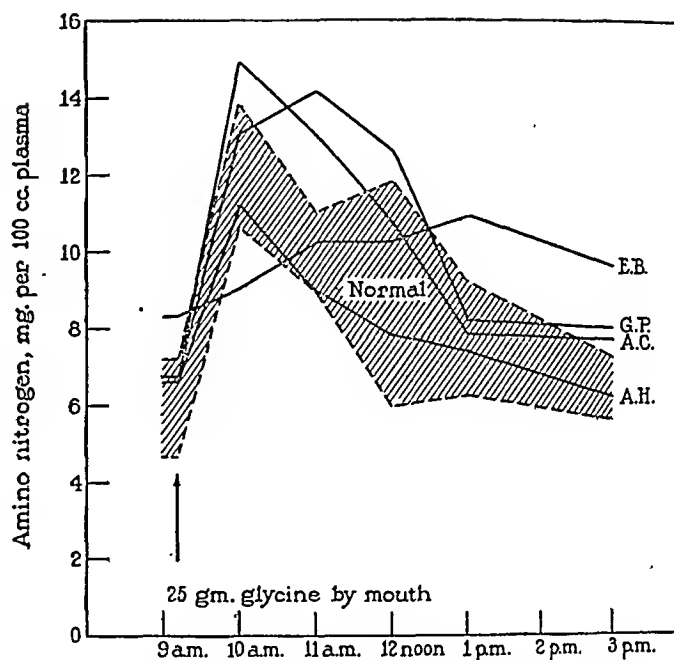


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The plasma amino nitrogen curves obtained in 4 patients with marked nephrotic syndrome accompanying the chronic active stage of hemorrhagic Bright's disease are illustrated in Figure 2. These patients still had urea clearances above 20 per cent of normal, and hence would not be classed as terminal (Van Slyke, Stillman, *et al.*, 1930). The postabsorptive plasma amino nitrogen curves fall nearly within the area which includes the curves obtained in the normal individuals.

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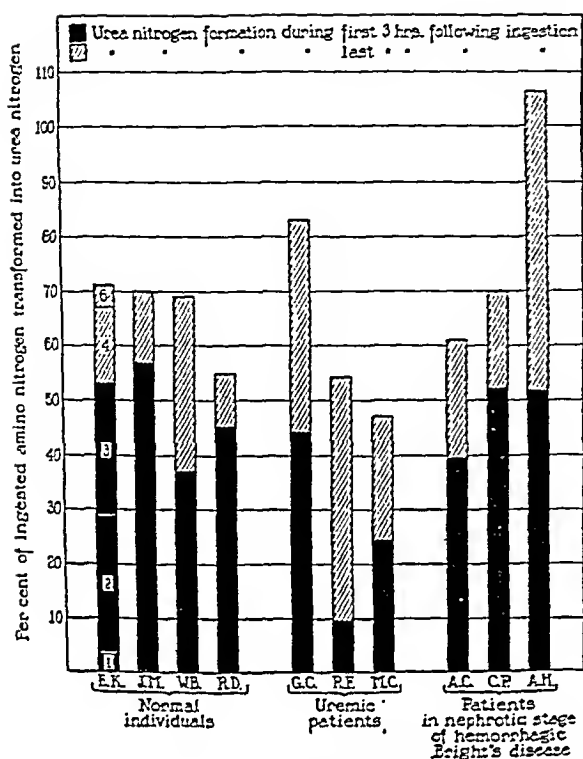


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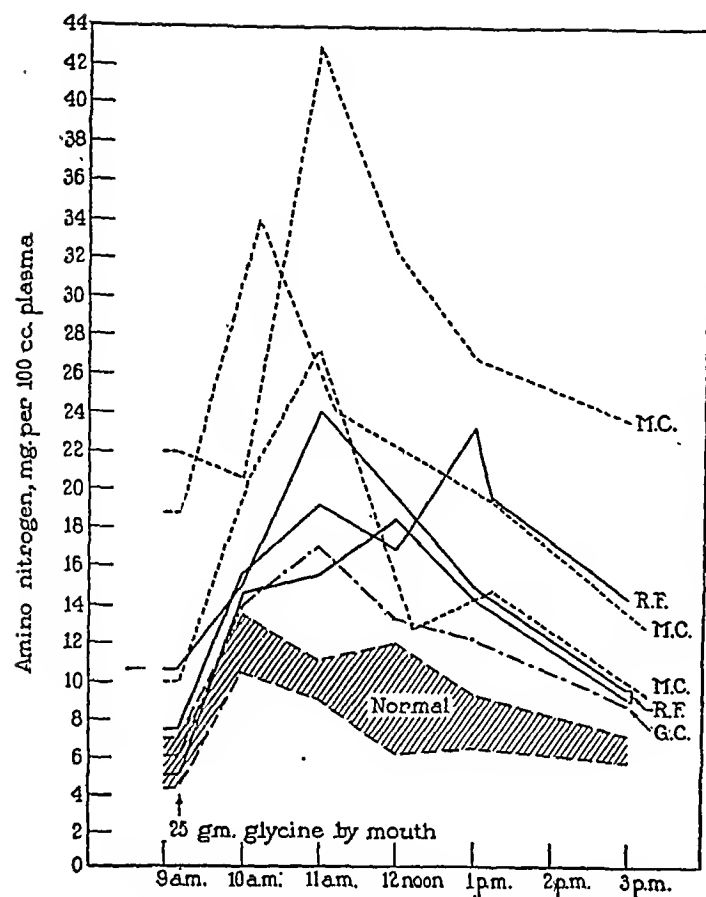


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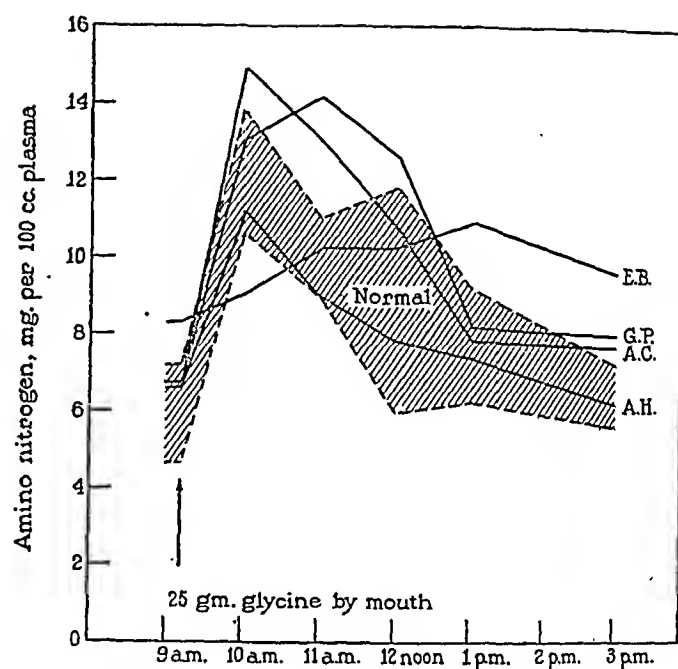


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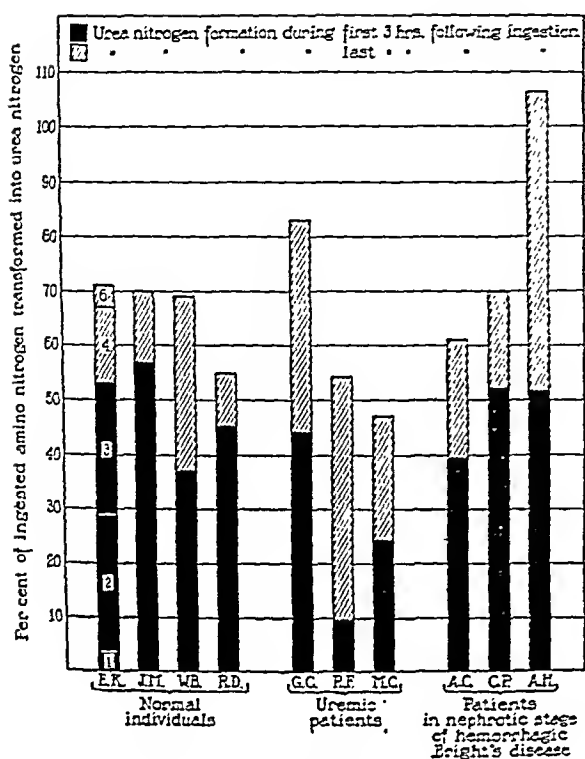


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ammonia into urea is unimpaired in uremia. They give support to the assumption that the delay in urea formation, observed after glycine ingestion in uremic patients, is caused by impairment in the body mechanism which deaminizes amino acids, rather than in the mechanism which forms urea out of the ammonia yielded by deaminization.

#### DISCUSSION

The experimental data presented above have revealed a distinct metabolic dysfunction in patients suffering from uremia and have thereby confirmed the results reported in a previous paper (Kirk, (1933)). In addition it is shown that putting the deaminizing function under strain by the ingestion of 25 grams of glycine may reveal impairment of this function at a time when the fasting plasma amino nitrogen is still within the normal limits.<sup>5</sup> No definite conclusion can be drawn from this investigation about the site of this impaired function, but it should be mentioned in this connection that all the uremic patients studied showed a normal liver function when subjected to the bromsulphalein test of Rosenthal and White (1925). Normal elimination of the dye was found even in a semicomatose patient, who on the day of the test had a fasting plasma amino nitrogen value of 38 mgm. per cent.

From clinical experience in this hospital it appears doubtful that demonstration, by the glycine feeding test, of a retarded rate of deaminization in a nephritic patient indicates the necessity for a low protein diet. The plasma amino nitrogen concentration usually returns to initial values within the 6-hour period of the test, which means that the deaminization, even of the rather large amounts of amino acid taken, is completed during the usual interval between meals. A review of the observed practical advantages of a normal protein intake in even the later stages of nephritis, based on the experiences obtained in this clinic, has recently been published by Alving (1934).

An illustration of the beneficial effect of free protein intake in face of nearly constantly elevated plasma amino nitrogen is seen in the clinical course of the disease of a nephritic patient, pre-

sented in Figure 1 of a previous paper (Kirk, (1933)). Amino nitrogen analyses of plasma were made daily for 3 months after recovery from an acute attack of uremia. Usually the fasting amino nitrogen values were found considerably elevated. In spite of these laboratory findings the patient was allowed an unlimited protein intake and on this regime gained weight and strength and was able to leave the hospital at the end of the period of observation with nearly completely restored working ability.

#### SUMMARY

1. A test of the deaminizing and urea-forming functions is described in which the plasma amino nitrogen curve and the amount of urea formed are observed during 3 hours after ingestion of 25 grams of glycine.

2. In uremic patients the increase in plasma amino nitrogen was 2 to 4 times greater than in normal individuals, and the return to pre-ingestion level was slower.

3. Normal persons transformed an average of 48 per cent of the ingested amino nitrogen into urea nitrogen during the first three hours following the glycine. In 2 out of the 3 uremic patients studied the urea formation in this period was reduced to 10 and 24 per cent respectively of the ingested nitrogen. However, during the period 3 to 6 hours after ingestion the uremic subjects formed as much urea as the normal subjects or more.

4. The same patients who showed high and prolonged curves for blood amino nitrogen and initially retarded urea formation after ingestion of glycine, were able to form urea from ingested ammonium citrate as rapidly as the normal individuals. The curve for blood ammonia following ingestion of 13.3 grams of ammonium citrate likewise showed no difference from the curve obtained in normal subjects. The conversion of ammonia into urea is therefore unimpaired in uremia. The retarded formation of urea observed after feeding of glycine appears to be attributable rather to delay in deaminization, than to delay in the subsequent transformation of the ammonia into urea.

5. In patients in the intermediate, chronic active stage of Bright's disease, no abnormality in

<sup>5</sup> For detection of impaired deaminization one amino nitrogen determination performed 3 hours after the ingestion of 25 grams of glycine suffices.

the curve for plasma amino nitrogen or in urea formation was found after feeding of glycine.

6. The finding of abnormally high curves for plasma amino nitrogen after ingestion of glycine, or even of moderately elevated content of plasma amino nitrogen in the fasting condition, does not apparently suffice to indicate the necessity for restricting protein intake.

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## OBSERVATIONS ON NORMAL SUBJECTS

Relying on the accuracy of the above method we determined to study in normal individuals the response of the plasma fatty acids to the intramuscular administration of adrenalin with the special purpose of combining such results with similar observations made on patients with disease of the liver. Such studies were suggested by the known high values for blood lipids in many cases with hepatic disturbances and were in progress before the publications of Himwich and his collaborators. It is of incidental interest that our results on normal individuals apparently confirm the qualitative findings made by these investigators although the quantitative changes are of much smaller magnitude. In normal subjects we have obtained what we believe to be significant increases in blood fatty acids after the injection of the adrenalin in every instance but one. The results are here reported and discussed.

*Experimental results*

The subjects were normal medical students or members of the hospital or laboratory staff. Control specimens of venous blood were taken about 14 hours after the last meal with the subject at rest, and 0.5 cc. of a 1 to 1000 solution of adrenalin (Parke, Davis and Co.) was then injected intramuscularly. Subsequent samples of venous blood were taken at intervals of one-half hour, one hour, and two hours. The subject remained at rest during the entire period. Blood sugar estimations as well as fatty acid determinations were made, and gave the usual post-adrenalin rise and fall. The results of the fatty acid determinations are shown in Table II.

A graphic representation of the average curve of fatty acid values obtained in this group of normal individuals is given in Figure 1. It will be noted that the maximum rise in fatty acids is reached in most instances at the end of half an hour after the injection of adrenalin, although in a few subjects the maximum rise seemed to come after one hour. The average increase above the fasting level was 30 mgm. per 100 cc., with a maximum rise of 69. In all but one instance the rise exceeded 12 mgm. but in one subject there was no appreciable rise. It will be observed that the only suggestion of a drop below the fasting

TABLE II

*Changes in blood fatty acid noted in normal individuals following the intramuscular injection of 0.5 cc. adrenalin (1:1000 solution).*

Subject	Fasting	½ Hour	1 Hour	2 Hours
	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.
D. C.....	270	286	320	270
B. B.....	294	298	298	271
H. H.....	259	280		244
C. M. J...	296	320	277	285
A. M.....	257	295	241	270
R. M.....	330	348	330	
M. R.....	271	329	352	282
G. T.....	283	291	296	293
J. W.....	263	291	278	275
T. U.....	326	362	325	333
T. Y.....	302	363	285	266
H. C.....	319	388	343	336
A. K.....	419	437	488	424

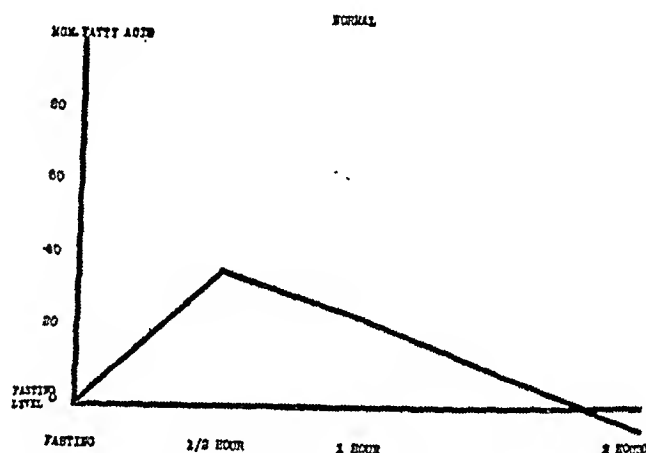


FIG. 1. AVERAGE NORMAL CURVE SHOWING RISE FROM FASTING LEVEL OF PLASMA FATTY ACIDS AFTER INJECTION OF ADRENALIN IN A GROUP OF NORMAL INDIVIDUALS.

level was at the two hour determination. At the end of two hours the fatty acid values returned approximately to a fasting level in nearly every instance. The fasting level of the plasma fatty acids varied between 203 and 419 mgm. per 100 cc.

*Discussion*

As far as the accuracy of the above observations is concerned it would seem to us that there exists little reason for doubt. The findings were consistent in the normal individuals studied, and the rise in fatty acid coincided with the known rise in blood sugar resulting from the injection of adrenalin. The accuracy within narrow limits of error ( $\pm 5.0$  mgm.) of the Stoddard and Drury method for the determination of fatty acids has already been commented on and is attested by



our own observations and by those of Long and Venning.

Granted that the methods used were accurate within the limits already noted ( $\pm 5.0$  mgm.), we believe our findings constitute acceptable evidence that normally the fatty acid content of the blood rises appreciably and rather rapidly following the intramuscular injection of adrenalin in man. That the rise is within much narrower limits than those reported by Himwich and his collaborators may best be explained by the error inherent in the method used by them for the determination of fatty acids, and also by some difference in the amounts of adrenalin used. Our results, nevertheless, support their conclusions that adrenalin temporarily increases the blood lipids.

The discrepancy between our results and those reported by other investigators who found no rise or an actual fall in fat values after the injection of adrenalin is less easy to explain. It is not necessarily probable that the difference lies in the fact that most of their observations were on animals which might react in another manner than man. Many of the observations were made on anesthetized or decerebrate animals, however, and this may have altered an otherwise normal sympathetic response.

Furthermore, the observations of other investigators, in most instances, were made at much longer intervals than one-half hour and one hour after the injection of adrenalin, and for this reason an early rise might have been missed. In some instances the adrenalin was given by continuous intravenous administration over a period of hours and in one set of experiments it was administered subcutaneously. Such variations in the administration of the drug may well have altered the type of response obtained.

Another important consideration arises in relation to the fact that other investigators for the most part confined their studies to animals. Inasmuch as the phenomenon under discussion is essentially a sympathetic response, it should be expected that any emotional disturbance could produce similar blood changes in a very short time. Disturbance of the animal during the period of early anesthesia or during the handling of the animal might evoke a discharge of adrenalin with resulting change in blood values in a few

minutes. In some observations on rabbits (14) we found it extremely difficult to avoid fictitious high resting values for blood sugar and fatty acids because of the apprehension the animals experienced during the withdrawal of blood. The resulting fatty acid curve in such instances not only failed to rise after the injection of adrenalin but at times fell, due, we believe, to the fact that the initial emotional response of the animal had already raised the fatty acid values to a high level. Only in a few well trained rabbits, in which pentobarbital was very carefully employed to quiet them, did we obtain curves similar to those reported in Table II.

That this response of the fatty acids can occur very rapidly is shown by the fact that frequently in human beings we have noted an almost maximal rise within fifteen minutes after the injection of adrenalin. If we regard the response as in a sense an emergency one, and comparable with the other well-known effects of adrenalin it is not unreasonable to expect it to reach its maximum in a relatively short period of time. From such considerations we believe our findings are not necessarily inconsistent with those obtained by previous investigators.

The physiological significance of the rise in blood fatty acids is not clear, but the suggestion obviously arises that it may be associated with the rapid mobilization of energy-producing material either for immediate consumption or for storage elsewhere to replace material which has been rapidly utilized.

#### OBSERVATIONS ON PATIENTS WITH HEPATIC DISEASE

As already mentioned it has been known for some time that in many patients suffering from various forms of hepatic disorders the level of the plasma lipids is considerably elevated above normal. The exact mechanism underlying such an elevation of blood lipids is not understood. Because of this fact we determined to obtain a series of fatty acid curves in patients suffering from various diseases of the liver. It seemed that a comparison of the results obtained with those already noted in normal individuals might provide further information regarding the mechanism involved in the normal rise in fatty acids.



following the intramuscular injection of adrenalin and might also provide a means of studying disturbances of hepatic function. The results of these studies are given below.

### *Clinical material*

The clinical material subjected to investigation included various types of liver disease and was selected from ward and private patients. In the 43 patients studied, the ultimate outcome is known in every case and in many instances the individual patients have been followed closely for more than three years. In general, it may be stated that the patients were suffering from acute infectious or toxic hepatic disorders, such as catarrhal jaundice or cinchophen poisoning, or from chronic disturbances, such as are encountered in the various forms of cirrhosis. The diagnosis was usually obvious, and in one-third of the cases was confirmed by operation or by necropsy.

### *Methods of study*

A curve of fatty acid values was obtained following the intramuscular injection of adrenalin, as already described. The patients were at rest in bed throughout the full two-hour period. In most instances a quantitative determination of the plasma bilirubin was made by the method of van den Bergh and a bromsulphalein retention test was performed as a further means of studying hepatic dysfunction and for comparison with the results of the blood lipid studies.

### *Results*

The curve obtained from a study of normal individuals has been shown in Figure 1. As already noted its configuration indicates a consistent tendency in normal individuals to a definite rise in blood fatty acids after the intramuscular injection of adrenalin, with a subsequent return to the fasting level. Because of the known high fasting values for plasma lipids in hepatic disorders it seemed wise to determine, if possible, whether the existence of a high fasting level of blood fatty acids would affect the character of the response to adrenalin. Observations were made on three patients with chronic nephritis with high fasting values of blood fatty acids. The results are shown in Figure 2 and may be com-

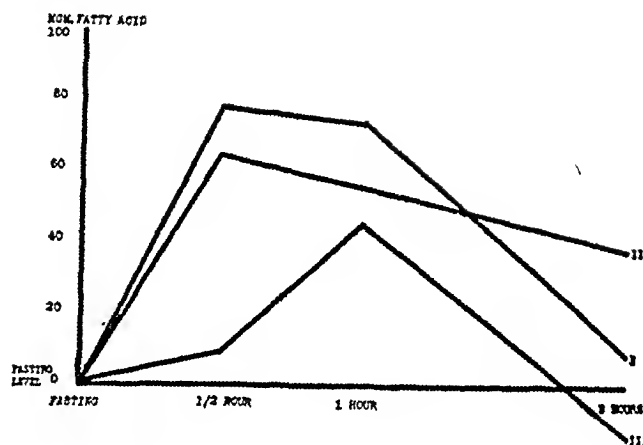


FIG. 2. FATTY ACID CURVES OBTAINED IN PATIENTS WITH CHRONIC NEPHRITIS.

The fasting level differs in each instance but for comparison graphs are drawn with O as the fasting level.

Fasting level values: I—Nephrosis = 607 mgm.

II—Nephrosis = 511 mgm.

III—Uremia, chronic glomerular nephritis = 363 mgm.

pared with the so-called normal curve in Figure 1. The fasting values were 607, 511, and 363 mgm. respectively. In the case represented by Curve III, Figure 2, starvation, a known cause of an increased value for plasma lipids, was added to the known existence of a nephritis. It will be noted that in each instance the curves obtained showed a marked rise to a peak after the injection of adrenalin and a subsequent fall approximately to the previous fasting level.

The curves presented in Figure 3 were obtained from two patients who were recovering from acute toxic liver injury. In both instances the "control" curve was obtained while the patients were on a low fat diet. The curves obtained after the administration of a high fat diet for a period of three days are given for comparison. Although the fasting level of the blood fatty acids was appreciably higher after the change in diet there still was noted a sharp rise in the blood lipids following the administration of adrenalin.

From the above observations, it would seem that an abnormally high fasting level of the blood fatty acids when due to a high fat intake, or to conditions such as exist in starvation or in nephritis, does not prevent a rise in fatty acids following the intramuscular administration of adrenalin, with a subsequent fall to the fasting level. Even under such conditions this response to adrenalin resembled that obtained in normal individuals.

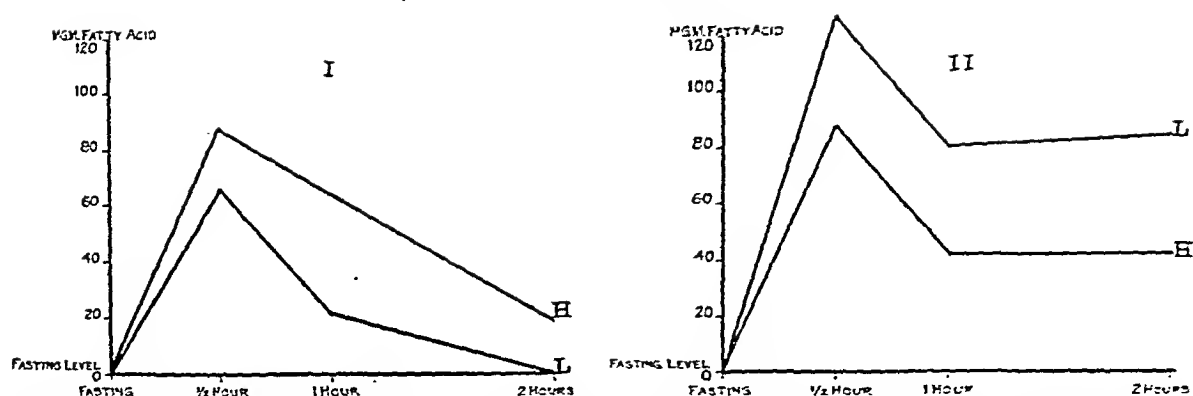


FIG. 3. FATTY ACID CURVES OBTAINED ON TWO PATIENTS WITH TOXIC JAUNDICE (I) ANTIMONY POISONING: (II) ARSPHENAMINE POISONING

Curves marked H obtained during the feeding on high fat diet. Curves marked L obtained during feeding on low fat diet. As in previous charts the fasting levels are charted at identical points although they actually represent different values as given above.

I. Fasting level high fat diet = 563 mgm.

Fasting level low fat diet = 550 mgm.

II. Fasting level high fat diet = 551 mgm.

Fasting level low fat diet = 466 mgm.

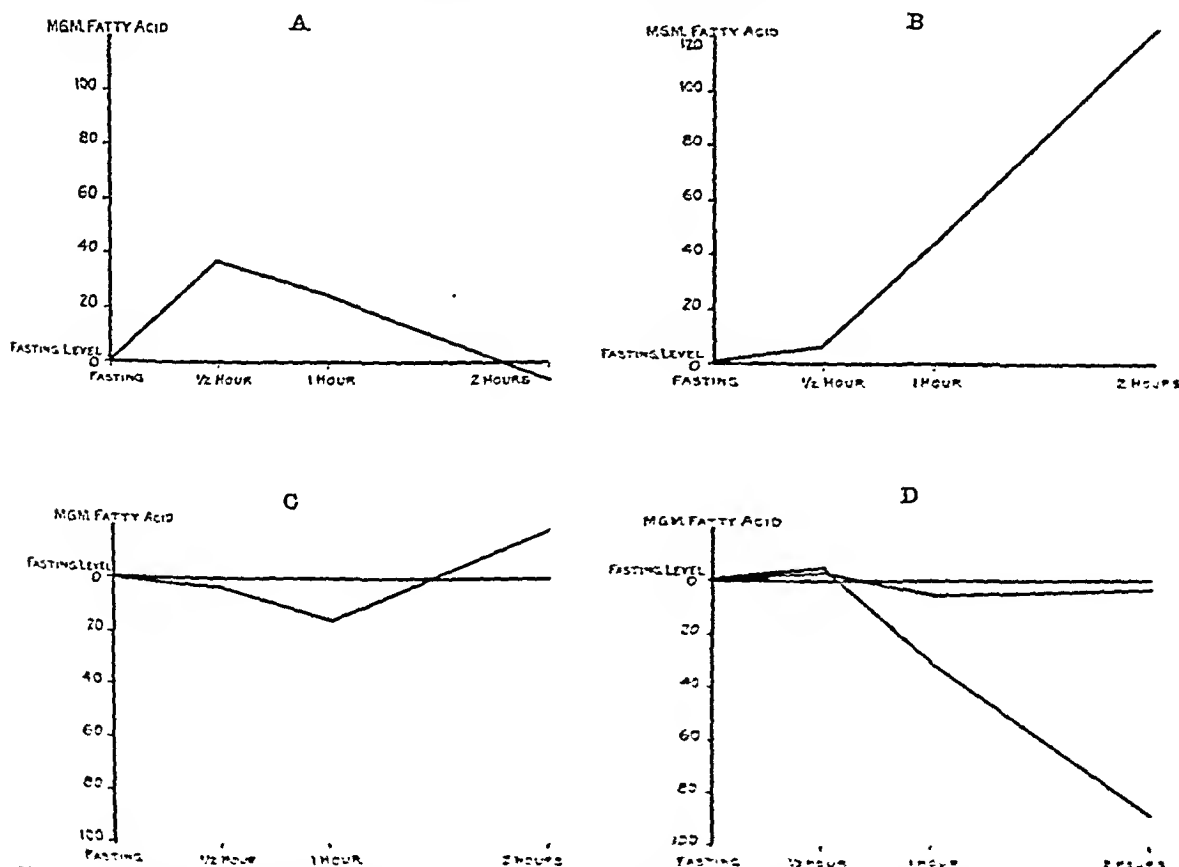


FIG. 4. TYPES A, B, C, AND D OF FATTY ACID CURVES AS OBTAINED AFTER INTRAMUSCULAR INJECTION OF ADRENALIN IN NORMAL PATIENTS AND IN PATIENTS WITH LIVER DISEASE. (See text.)

Two D Curves are given, one showing no appreciable change from fasting level and the other showing the continuous fall.

It seems reasonable to assume, therefore, that the high fasting fatty acid values encountered in diseases of the liver would not in themselves alter the response to adrenalin.

We have divided the curves obtained from the group of 43 patients with various types of hepatic disease into three arbitrary types and have classified them for comparison with the normal as follows (see Figure 4):

"A" (normal) Curve—This curve is characterized by an appreciable rise which usually appeared within one-half hour after the administration of adrenalin, and a return to approximately a fasting level at the end of two hours.

"B" Curve—A curve showing no initial fall

in fatty acids after the administration of adrenalin, but a rather continuous rise of at least 15 mgm. per 100 cc. within two hours, with little if any terminal drop.

"C" Curve—A curve showing a fall in the fatty acids below the fasting level, followed by a rise to at least the fasting level.

"D" Curve—A curve showing no rise or fall of more than 5 mgm. from the fasting level, or a continuous fall.

### Results

We have grouped our results in relation to such a classification of the various curves obtained. The findings are tabulated in Table III together

TABLE III

Data obtained from cases of hepatic disease following the intramuscular injection of 0.5 cc. adrenalin (1 : 1000 solution)

Patient number	Sex	Age years	Diagnosis	Bilirubin mgm. per 100 cc.	Bromsulphalein retention per cent	Fasting fatty acid mgm. per 100 cc.	½ Hour fatty acid mgm. per 100 cc.	1 Hour fatty acid mgm. per 100 cc.	2 Hour fatty acid mgm. per 100 cc.	Type fatty acid curve	Duration of life after tests	Remarks
1	M.	23	Biliary cirrhosis*	4.5	50	320	326	324	339	B	12 mos.	Living. Light work.
2	M.	47	Toxic cirrhosis			327	392	380	388	B	12 mos.	Living. Working.
3	M.	55	Toxic cirrhosis: cinchophen	3.5	30	290	304	300	264	B	13 mos.	Semi-invalid.
4	M.	59	Toxic cirrhosis: alcohol	1.5	40	273	291	285	283	A	14 mos.	Living. Working.
5	F.	14	Toxic cirrhosis	4.2	35	233	190	220	210	D	15 mos.	Chronic invalid. Died acute infection.
6	M.	35	Toxic cirrhosis	6.8	0	385	363	351	357	D	15 mos.	Living. Fatigues very easily.
7	F.	50	Biliary cirrhosis	4.9	50	492	460	462	436	D	17 mos.	Dead. Invalid while alive.
8	F.	47	Toxic cirrhosis: cinchophen	4.8	55	374	395	381	371	A	21 mos.	Living. Well.
9	M.	54	Cirrhosis: syphilis, arsenic	4.7	25	322	322	325	338	B	24 mos.	Dead from carcinoma esophagus.
10	F.	33	Biliary cirrhosis*	23.0	50	2020	1710	1588	2032	C	24 mos.	Dead. Invalid while alive.
11	M.	51	Cirrhosis			253	280	254	243	A	2 yrs.	Living. Fairly well.
12	F.	23	Toxic cirrhosis	3.5	0	301	380	—	345	B	25 mos.	Living. Fairly well.
13	M.	46	Cirrhosis: syphilis	0.5	0	247	273	253	246	A	2 yrs.	Living. Working.
14	F.	55	Toxic cirrhosis: antimony	23.0		501	578	561	537	B	6 mos. 3 yrs.	Living. Fair health.
15	F.	32	Toxic cirrhosis: pregnancy*†			441	451	488	576	B	5 mos. 3 yrs.	Living. Fair health.
16	F.	39	Toxic cirrhosis: arsenic	19.7	100	421	548	501	506	B	5 mos. 3 yrs.	Living. Fairly well.
17	F.	40	Toxic cirrhosis	3.5	0	260	288	346	291	B	3 yrs. 8 mos.	Living. Fairly well.
18	F.	65	Biliary cirrhosis: post- cholelithiasis	1.8	15	276	280	305	281	A	4 yrs.	Living. Well.
19	M.	31	Infectious jaundice	21.7	0	472	487	478	462	A	4 yrs.	Living. Well.
20	M.	45	Infectious jaundice	20.8		330	378	352	341	B	1 mo.	Living. Well.

\* Confirmed at operation.

† Confirmed at autopsy.

‡ Five months pregnant.

TABLE III—Continued

Patient number	Sex	Age	Diagnosis	Bilirubin	Bromophthalein retention	Fasting fatty acid	1/2 Hour fatty acid	1 Hour fatty acid	2 Hour fatty acid	Type fatty acid curve	Duration of life after tests	Remarks
		years		mgm. per 100 cc.	per cent	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.			
21	M.	46	Toxic cirrhosis: alcohol	0.5	50	338	365	373	379	B	4 yrs. 4 mos.	Dead. Well until acute terminal infection.
22	F.	23	Infectious jaundice	15.6	25	608	659	610	601	A	4 yrs. 4 mos.	Living. Well.
23	F.	37	Toxic cirrhosis*	0.5	20	547	504	435	446	D	4 yrs. 4 mos.	Living. Fatigues very easily.
24	M.	48	Acute yellow atrophy: —Cause?	15.0	100	271	271	249	252	D	1 day	Dead
25	M.	45	Toxic cirrhosis:† alcohol	6.6	40	198	188	202	190	C	2 days	Dead
26	M.	35	Toxic cirrhosis: alcohol	1.5	25	286	292	272	269	D	5 days	Dead. Acute yellow atrophy.
27	M.	66	Cancer of pancreas*	19.8		380	376	398	383	B	10 days	Dead. Postoperative complication.
28	M.	42	Acute yellow atrophy; pylephlebitis?	18.0	100	323	318	311	312	D	10 days	Dead.
29	M.	63	Carcinoma of pancreas*†	16.3	100	453	444	420	415	D	12 days	Dead. 9 days post-operative.
30	F.	48	Carcinoma bile ducts†	30.0		1336	1341	1298	1310	D	16 days	Dead. Cholemia.
31	F.	67	Toxic cirrhosis:† cinchophen	4.8	70	383	362	356	351	D	3 wks.	Dead. Cholemia.
32	M.	45	Pylephlebitis: post-cholecystectomy	2.0	25	364	355	317	302	D	3 wks.	Dead. Toxemia.
33	F.	50	Biliary cirrhosis†	23.4	100	404	396	381	343	D	1 mo.	Dead.
34	M.	20	Biliary cirrhosis	6.0	55	242	239	227	260	C	6 wks.	Dead.
35	F.	48	Toxic cirrhosis	3.4	60	327	368		332	A	3 mos.	Dead. Acute hemorrhage.
36	F.	38	Biliary cirrhosis*	6.6	35	418	426	422	422	D	3 mos.	Dead. Cholemia.
37	F.	37	Toxic cirrhosis*	2.5	35	270	262	277	274	C	3 mos.	Dead. Cholemia.
38	M.	49	Toxic cirrhosis: alcohol	3.8	35	475	479	444	387	D	3 mos.	Dead. Cholemia.
39	M.	54	Toxic cirrhosis: alcohol	2.0	70	242	213	204	159	D	6 mos.	Dead. Acute yellow atrophy.
40	M.	26	Syphilis	22.5	100	679	604	602	568	D	7 mos.	Dead.
41	M.	30	Biliary cirrhosis*	15.5	35	619	608	636	681	C	7 mos.	Dead. Cholemia.
42	M.	62	Biliary cirrhosis*	24.0	100	489	492	484	486	D	7 mos.	Dead. Acute yellow atrophy.
43	F.	32	Toxic cirrhosis†	5.6	65	266	255	274	290	C	8 mos.	Dead. Pneumonia after exploration.

with certain other laboratory and clinical data. In each instance we have given the actual values obtained for the variations in blood fatty acid, and have also classified the curve according to the above arbitrary standards.

### Discussion

These findings suggest that the character of the fatty acid curve obtained after the intramuscular injection of adrenalin in man tends to be definitely altered in conditions characterized by liver damage. The course of the curve, as has been noted, seems to be independent of the initial level of the fatty acids. Of interest, clinically, is the possibility of prognostic value attaching to

such curves in the presence of liver disturbances. Of the 43 patients studied 18 are living, with a survival period of from 8 to 52 months after the test was made. Of this group of 18 living individuals, 16 had either A or B curves. Of the 25 patients who died 21 had either C or D curves. (See Table IV.)

Of the 20 patients with A or B curves all but two survived for more than a year after the observations were made. The average survival period was 30 months with 5 patients living for more than 4 years. One of the group, Patient 27, lived only ten days after his blood was studied. He died of bronchopneumonia four days after a cholecystogastrostomy for carcinoma of the liver.

TABLE IV

*Tabulation of fatty acid curves in relation to survival and state of health*

Type of curve	Number of cases well	Number of cases invalid	Number of cases dead
A.....	6	1	1
B.....	2	7	3*
C.....	0	0	6
D.....	0	2	15

\* Cancer esophagus, cellulitis of leg, and postoperative death, respectively.

of the pancreas. The other case, Patient 35, died from a ruptured esophageal varix two months after the fatty acid determinations. Of these two deaths, one at least could hardly be attributed to hepatic insufficiency.

Two cases, Patients 9 and 21, with A or B curves died 29 and 52 months after the test was performed. During the entire survival period there were no symptoms referable to the underlying disease of the liver, and death occurred as a result of cancer of the esophagus and cellulitis of the leg respectively.

Of the 23 patients presenting C or D curves 18 died within 8 months of the observations following the injection of adrenalin. The average survival period of the group was 7 months, with 9 patients dying within a month of the test. Of the 5 patients with C or D curves who survived more than eight months, three died after 15 months, 17 months and 24 months respectively. Their course was one of almost continuous invalidism.

A comparison of the relation existing between the type of curves obtained and the degree of liver damage as measured by standard liver function tests or by clinical findings made at the time of the fatty acid determinations is made in Tables V and VI.

TABLE V

*Comparison between (1) type of curve obtained, (2) serum bilirubin, and (3) bromsulphalein retention*

Type of curve	Number of cases	Mean serum bilirubin	Mean bromsulphalein retention
		<i>mgm. per 100 cc.</i>	<i>per cent</i>
A.....	7	7.0	28.0
B.....	7	9.0	36.4
C.....	6	9.8	46.5
D.....	16	10.9	59.4

TABLE VI

*Comparison between the type of curve obtained and the presence of edema and ascites*

Type of curve	Number of cases	Number of cases showing ascites	Number of cases showing edema
A and B....	20	6	6
C and D....	23	13	11

It seems obvious, from these findings, that not only do those patients with A and B curves have a much longer survival period than those with C and D curves, but in addition such curves are associated with less marked evidence of serious liver damage as measured by the usual clinical and laboratory methods.

Although the variations observed in the fatty acid curves correspond in general to the apparent severity of the hepatic disturbance as measured by the presence of edema or ascites, or by the intensity of the jaundice and the degree of dye retention, this is not true in every individual case. In several patients the severity of the liver damage, as indicated by the other laboratory tests or by clinical findings failed to be reflected in the type of fatty acid curve. In such instances the subsequent course proved that the fatty acid curve was the best guide to prognosis. The findings in Patient 8 illustrate this. In this case the patient had been jaundiced for two months after prolonged administration of cinchophen. On admission she showed moderate jaundice, emaciation, purpura, ascites, marked edema, and bilateral pleural effusion. The liver and spleen were both enlarged, and the clinical picture was that of a very severe liver toxemia, probably with subacute yellow atrophy. The bilirubin was 4.8 mgm. per 100 cc., the bromsulphalein retention was 55 per cent at the end of 30 minutes, and the red blood count was only 3,900,000 cells per cu. mm. The urine was heavily bile stained and the stools were clay colored. In spite of such obvious severe liver damage the fatty acid curve was normal in shape. Under proper treatment recovery was unusually prompt, and the patient is now working full time 30 months after the test was performed. The prognosis given by several observers was unfavorable, but actually the patient did extremely well.

An example of the opposite situation is also of interest. Patient 32, ten days after a chole-

cystectomy began to run a slightly elevated temperature, and became very slightly jaundiced. Physical signs, with the exception of the slight jaundice revealed nothing. The serum bilirubin was 1.9 mgm. per 100 cc. and the bromsulphalein retention was 35 per cent. Ten days later the clinical condition of the patient apparently was unchanged but the bromsulphalein retention had dropped to 20 per cent, suggesting definite improvement. A fatty acid curve done at this time showed a typical D curve. In spite of the apparent improvement, as indicated by the better dye excretion, the patient became progressively worse from this point in the disease, and died in three weeks. Autopsy showed multiple small areas of infection and necrosis throughout the liver.

From such findings it would appear that a correct interpretation of the fatty acid curve offers a valuable method for prognosis, as compared with other laboratory tests or clinical findings. Examination of our results reveals the fact that in only one instance, Patient 35, was an A curve associated with a short survival period. Moreover, in this instance death occurred from an acute hemorrhage, presumably from esophageal varices, rather than from liver insufficiency. Only two patients with D curves failed to do poorly. One, Patient 6, is a semi-invalid, and has been limited in his activity much of the time during his fifteen months survival. The other, Patient 23, still survives after more than four years, and while now a semi-invalid, would seem to be a distinct exception to our prognostic rule.

It seems to us that a determination of the blood fatty acid curve after the intramuscular injection of adrenalin offers prognostic aid in patients with liver disease, and apparently regardless of the type of hepatic involvement. As with every other laboratory test there are exceptions to the rule, but we believe these will be few in number, and will not seriously reduce the value of the test. We suggest that the test may be of particular importance as an aid to prognosis in those acute or subacute conditions involving the liver in which other observations seem to indicate very serious involvement of the organ, and it is of particular interest that apparently important deductions may be suggested by a single test. In this respect it would seem to have an advantage over other lab-

oratory procedures where repeated observations seem to be necessary in order to obtain information of prognostic importance. From our findings we are inclined to believe that according to our arbitrary classification A and B curves indicate a relatively excellent prognosis even in the face of apparently severe liver injury provided adequate treatment is instituted; C and D curves suggest a prognosis of chronic invalidism or of a relatively short survival period.

The blood sugar findings corresponding to the fatty acid curves are not given in detail, but corresponded to those reported by Loeb, Reeves and Glasier (15). They seemed to show no constant changes consistent with the underlying hepatic condition.

#### SUMMARY

1. We have studied the effect of the intramuscular injection of adrenalin upon the blood fatty acids in thirteen normal human beings.

2. Our observations show an almost constant type of response which consists in a fairly sharp but moderate rise in the plasma fatty acids within about one-half hour after the injection of the drug followed by a subsequent return nearly to the fasting level in about two hours.

3. We have presented evidence that in patients suffering from disorders of the liver there is an abnormal response of the blood fatty acids to the intramuscular injection of adrenalin.

4. We suggest that this abnormal response of the blood fatty acids may be used as a valuable guide to prognosis in patients with liver disease.

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# THE EXPERIMENTAL PRODUCTION OF LOSS OF HEMATOPOIETIC ELEMENTS OF THE GASTRIC SECRETION AND OF THE LIVER IN SWINE WITH ACHLORHYDRIA AND ANEMIA

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The mode of origin of pernicious anemia and of conditions symptomatically allied to it, sprue and tropical macrocytic anemia, has been the subject of detailed and extensive investigation during the last decade. Although considerable advances have resulted from clinical studies in spite of limited material, one of the chief handicaps to more rapid progress has been the difficulty of reproducing similar diseases in animals. Certain isolated pathological changes including glossitis, macrocytic anemia and achlorhydria, similar to those of pernicious anemia, have been caused to occur independently, but in no reported instance have the major pathological and physiological alterations of pernicious and allied anemias been produced as a disease syndrome in a lower animal species.

The present communication deals with the results of an attempt to cause in swine a symptom-complex which might be etiologically, symptomatically, therapeutically and pathologically similar to that characterizing the group of macrocytic anemias of man. The condition studied was brought about by the administration of a specially devised diet, and was marked by hematological, lingual and gastro-intestinal changes similar to, though not entirely identical with, those of pernicious anemia and of conditions allied to it.

To comprehend thoroughly the rationale of the experiments to be reported, a brief review is required of the conditions in human beings which it was desired to simulate.

Pernicious anemia, the anemia of sprue, and tropical macrocytic anemia, present hematological changes which are, in a general way, similar. A further likeness lies in the fact that all three may be alleviated by the administration of whole liver or of a suitably prepared extract. Distinct differences exist, however, among which are the dissimilar effects following the administration of a lysate of brewers' yeast. Whereas the symp-

toms of tropical macrocytic anemia are regularly alleviated by the administration of that material, favorable results are only occasionally obtained in sprue and in pernicious anemia. If, in the latter two conditions, however, the administration of the yeast extract is supplemented by the administration of normal gastric secretion, which, by itself, is inactive, the combination is therapeutically active (Strauss and Castle (6)). The most plausible explanation of the foregoing facts is that all three symptom-complexes are due to the same fundamental cause—a lack of some substance such as that contained in the yeast cell. Such a lack may be a simple dietary insufficiency, as in the case of tropical macrocytic anemia, or it may be an insufficiency, existing even in the presence of adequate dietary intake, due to the inability of the body to utilize the substance. In pernicious anemia, and in certain cases of sprue, it would appear that an as yet unidentified gastric ferment is required for the conversion of the principle in the yeast to a hematopoietic factor.

Clinical observation suggests that in both sprue and pernicious anemia an early stage in the sequence of pathological events is an inflammatory change of the mucous membranes without anemia. This is manifested visibly by glossitis, stomatitis, proctitis or vaginitis, and symptomatically by oesophageal and gastro-intestinal pain and distress and by diarrhea. This first stage is not necessarily associated with either achlorhydria or determinable hematological disturbance. As the disease progresses, the changes affecting the mucous membranes become less acutely inflammatory and more atrophic in nature. Gastric achlorhydria, loss of hematopoietic activity of the gastric juice, and anemia, may develop in the order named. Experimental studies of sprue by Castle and Rhoads (1) established the fact that the acutely inflammatory changes in the mucous membrane are quite as susceptible to cure by the adminis-



tion of yeast extract as is the hematological syndrome of macrocytic anemia. It seems probable, therefore, that the inflammatory lesion of the mucous membrane is due to a lack in intake of, or an inability to utilize, some constituent of yeast. It is inferred, from the symptomatology, that the lesions of the mucous membrane seen in the oral cavity are simply visible examples of similar lesions throughout the gastro-intestinal tract. The conclusion is drawn that these changes so alter the gastric mucosa that it is unable to secrete a ferment capable of converting the essential principle of yeast extract into a substance required to effect hematopoiesis.

If this hypothesis is true, a method of reproducing the pathological states under discussion is apparent. Diets should be so arranged as to omit the required constituent of brewers' yeast. Unfortunately, such a procedure is impossible at present because the identification of that constituent has not been achieved. Hence, artificial diets, lacking only that substance, cannot be supplied. Another experimental approach exists, however, in the study of those pathological states in animals which may be prevented or cured by that fraction of brewers' yeast which is curative in man. The significance of such experiments would be more definite if the condition in the animal presented some symptomatic similarity to the disease of human beings under discussion. Such a condition is at hand in canine black-tongue.

The literature bearing on the subject of black-tongue in dogs has been recently reviewed by the authors (2) and need not be discussed here. It suffices to state that, as originally described, it is an acute disease, characterized by stomatitis, glossitis, salivation, diarrhea and prostration. It is nearly always fatal if untreated, or if treated late. A decrease in the number of circulating polymorphonuclear leukocytes is frequently a terminal feature (3).

As shown by Goldberger and Wheeler (4), black-tongue can be produced at will in dogs by feeding a diet composed principally of corn meal, but also containing casein, sugar, cottonseed oil, cod liver oil, and a salt mixture. It may be prevented, or, if treated in the early stages, cured, by yeast or meat, or less certainly by a variety of foodstuffs, mostly of animal origin. The factor

preventing black-tongue is resistant to autoclaving. A rough parallelism was shown to exist between the distribution of the heat-labile, water-soluble, anti-neuritic vitamin B and the factor preventing black-tongue. The latter was thus held to be a part of the vitamin B complex, and, as such, it was tentatively identified with vitamin B<sub>2</sub>, a term later changed by certain authors to vitamin G.

The nature and action of vitamin B<sub>2</sub> (G) is still not completely clear. As applied to any thermostable fraction of foodstuffs possessing biological activity, it clearly may include a variety of substances of different composition and action. The accepted method of both qualitative and quantitative estimation of vitamin B<sub>2</sub> (G) is based upon the power of that vitamin to promote growth of young rats fed diets complete except for the thermostable factor. Direct comparison of the factor preventing black-tongue and the one promoting rat-growth has unfortunately received little study, and it is still not certain whether those two factors are or are not identical.

The authors (2) reported hematological studies of dogs in which chronic recurrent black-tongue was caused. This was effected by slight changes in the diet producing black-tongue, and by treating insufficiently the acute phase of the disease. In those animals an anemia of moderate degree appeared which was frequently macrocytic in type. In its response to oral therapy it presented certain points of similarity to the anemia of sprue and pernicious anemia. The pathological changes in the mouth, tongue and bone marrow resembled those of sprue and pernicious anemia. In certain respects, however, the likeness was not complete. The anemia produced in the experimental animal was not progressive, it was not constant, it was associated with neither hyperbilirubinemia nor achlorhydria, and it was not relieved by the parenteral administration of liver extract. Furthermore, it appeared that the dog, due to a certain characteristic of the species, was not suitable for the production of a pathology identical with that of the human disease.

We have discussed somewhat the mode of production of the gastric dysfunction which precedes the hematological changes of pernicious anemia. This dysfunction has been attributed to an insufficiency in intake or utilization of some constituent

of yeast. If this is correct, the similar changes in the mucous membrane of canine black-tongue should be followed by gastric achlorhydria and loss of the hematopoietic power of the gastric juice, since a similar fraction of yeast is prophylactic and therapeutic in that condition. Experimentally, this was not the case in the dog. Achlorhydria occurred, but only irregularly and as a late event. The hematopoietic power of the gastric juice could not be estimated because it was found that such a power is not possessed by the gastric secretion of normal dogs. Furthermore, Richter, Ivy and Meyer (5) showed that dried, defatted dog's stomach does not possess the hematopoietic effect which is present in high concentration in swine stomach. As would be expected if the anti-anemia effect of liver were due to a stored product dependent partly upon the activity of a gastric enzyme for its formation, Strauss and Castle (6) found canine liver to be low in anti-pernicious-anemia power. This fact has been confirmed by Richter, Ivy and Meyer (5).

This being the case, it was clear that by experiments on dogs one of the major aims of the study could not be attained, i.e., an induced inability of the animal stomach to secrete anti-anemic factor, since in the dog no considerable amount of that factor is present under normal conditions. It is present normally in swine, however, as shown by the employment of hog's stomach as a commercial preparation for the treatment of pernicious anemia. Hence, it was decided to continue the study, substituting the swine for the dog as an experimental animal.

#### REVIEW OF LITERATURE

The literature on the attempted experimental production of anemia in animals may be discussed under several headings.

Gastric dysfunction is a prominent etiological factor in pernicious anemia and in sprue; furthermore, the former has been reported to follow extirpation of the stomach in human beings. Elaborate experiments involving gastric resection in animals have been reported by Maisson and Ivy (7), and in their publications the literature bearing on the subject has been thoroughly reviewed and discussed. It suffices to state here that gastrectomy in rats, pigs and dogs results only in a hypochromic anemia which is curable by iron. Some other factor than the gastric is clearly required for the production of an anemia of the macrocytic type. That this other factor may have to do with the intestine was suggested by Maisson and Ivy (7).

and this suggestion is supported by the observations of Seyderhelm et al. (8). The latter produced a macrocytic anemia in dogs by causing a stenosis of the small intestine. That the extra-gastric factor is not wholly a lack of an intestinal secretion is suggested by unpublished experiments in this laboratory. All but a few centimeters of the ileum of dogs was resected with the resultant production of only a mild hypochromic anemia. Apparently, some disorder involving both the stomach and intestine is required to produce the desired result. This may explain the observations of Brown (9), who found that pernicious anemia was almost invariably associated with lesions of the gastro-intestinal tract, but that the lesions might be located at almost any level and might be of almost any type.

Experiments dealing with the production of nutritional anemia in animals have recently been reviewed by Davidson and Leitch (10). The microcytic anemias resulting from the feeding of diets poor in iron or copper are well known, but have little application to the problem under discussion. Both iron and copper deficiencies are associated with a greater reduction of hemoglobin than of erythrocytes, and are specifically relieved by the administration of the lacking element. The anemias found in man associated with the conditions under discussion are not so characterized. A hypochromic or microcytic anemia occurs in the experimental blood dyscrasias which depend for their production upon depletion of the available hemoglobin stores by bleeding. Experiments of that type have been exhaustively studied by Whipple and Robschtein-Robbins (11). The anemia produced was not entirely due to a lack of iron, although the administration of iron accelerated the production of hemoglobin. Some other factor was presumably involved, since the dogs had been fed a somewhat restricted diet, and certain foodstuffs added to the diet were effective, exclusive of their iron content, in causing increase of hemoglobin in the blood.

The work of McGowan and Sinclair (12) has a definite bearing upon the studies here presented. He observed that young pigs kept on a ration of corn, fish meal and draft became ill with anemia, damage to the liver, jaundice and changes in the bone marrow. The anemia was, in many instances, of the macrocytic type, and following the administration of raw liver reticulocyte rises occurred, with subsequent cure. They state that during the height of the anemia the femoral marrow was red and cellular. It became fatty once more as recovery proceeded. Moreover, gastric juice obtained at postmortem, in one instance was devoid of free hydrochloric acid. Unfortunately, no detailed studies of the composition of the diet, of the histology of the bone marrow, of the possible changes in gastric secretion, or of the comparative effects of various therapeutic methods, were reported.

The extensive literature dealing with the conception that canine black-tongue is to be regarded as experimental pellagra, and with the content of the factor preventing black-tongue in various foodstuffs, has been referred to and needs no review here. The effect on the gastric secretion of dogs from feeding diets deficient in vitamin B<sub>12</sub>

has been described by Cowgill and Gilman (13). Changes in the central nervous systems of dogs fed diets deficient in vitamin B<sub>2</sub> (G) rather than in anti-neuritic vitamin have been published by Zimmerman and Burack (14). Since the diet used in the experiments reported in this communication produced canine black-tongue, but was found to contain both of these essential food substances when tested on the rat, it is difficult to evaluate the pertinence of those previous studies in which there was an almost complete lack of the vitamin B fractions.

#### METHODS

Swine weighing from 11 to 13 kgm., and of both sexes, were used. The animals were kept in individual cages during the winter, but were in a single pen in the open air during the summer months; with this exception, the conditions were uniform. Shavings were employed as bedding. No attempt was made to prevent coprophagy. The animals were fed daily and were allowed to eat as much as they desired. The experimental diet used was a modification of the Goldberger and Wheeler (4) diet No. 123 which produces black-tongue. It consisted of the materials shown in Table A.

TABLE A  
*Composition of experimental diet*

Articles of diet	Quantity	Nutrients		
		Protein	Fat	Carbohydrate
	grams	grams	grams	grams
Corn meal.....	400	33.6	18.8	296.0
California black-eyed peas..	50	10.7	0.7	30.4
Casein (purified).....	60	52.0		
Cane sugar.....	32			32.0
Cottonseed oil.....	15		30.0	
Cod-liver oil.....	30		15.0	
Rice polishings.....	40			
Sodium chloride.....	3			
Calcium carbonate.....	10			
Total nutrients.....		96.3	64.5	358.4
Nutrients per 1000 calories..		40.1	26.9	149.3

In order to ascertain the effect of a deficiency of vitamins, other than of the water-soluble vitamin B complex, control experiments were performed. Swine similar to those used in the major experiment were kept under similar conditions and were fed a diet which was vitamin deficient, but was otherwise adequate. By adding suitable supplementary materials this diet could be made deficient in any single vitamin or combination of vitamins which it was desired to study. The basal diet used was the one recommended by Cowgill and Gilman (13) and consisted of the ingredients shown in Table B.

Erythrocyte counts, hemoglobin determinations, white cell counts and estimations of the mean corpuscular volume were made weekly as a routine and more frequently if indicated. All determinations were made on blood

TABLE B

#### *Composition of basal diet*

Casein (purified) .....	35.4 grams
Cane sugar .....	27.0 grams
Lard .....	21.6 grams
Bone ash .....	2.4 grams
Salt mixture (Mendel and Underhill) .....	2.4 grams
Water .....	12.2 grams

from the femoral vein. The erythrocytes were counted in the usual manner, using pipettes certified for accuracy by the U. S. Bureau of Standards. The hemoglobin determinations were made by the Sahli acid hematin method, using calibrated tubes and standards which were frequently checked by the Van Slyke (15) method of determining O<sub>2</sub> capacity. The mean corpuscular volume was calculated by the hematocrit method of Wintrobe (16). Reticulocytes were stained by allowing the wet blood smear to come in contact with dry brilliant cresyl blue stain. They were later counterstained by Wright's method.

Studies of the gastric secretion were made on all the animals at two-week intervals. Only two samples were examined, one obtained from the fasting stomach and a second withdrawn from 20 to 30 minutes after the intramuscular injection of histamine. These specimens were titrated in the usual way with 0.1 normal NaOH, using Töpfer's reagent and phenolphthalein as indicators. Control studies of the histology of the bone marrow of the experimental animals were made on tissue removed at operation from the femur.<sup>1</sup>

All of the swine were autopsied, at which time specimens of various tissues including the bone marrow were fixed in Zenker's fluid containing 5 per cent acetic acid as well as in a 10 per cent solution of formaldehyde in Zenker's fluid. The tissues fixed in the Zenker's fluid with acetic acid were stained with eosin and methylene blue. Material fixed by other fluids was stained by the Giemsa method as modified by Wolbach (17).

Gastric juice in amounts sufficient to allow it to be tested for its content of anti-pernicious-anemia substance was obtained from individual swine which had been allowed no food for twenty-four hours. A stomach tube was inserted and the fasting gastric content was removed and discarded. One cubic centimeter of a 1:1000 dilution of histamine was then injected intramuscularly and all the fluid secreted in response to that stimulus was collected. Great care was exercised to keep the gastric juice cold and strongly acid until it was used.

In preparing the mixtures of gastric juice and yeast extract, the juice was first filtered through fine gauze. Twelve grams of yeast extract (Vegex<sup>2</sup>) were then suspended in each 150 cc. of the fluid. The mixture was incubated at 37.5° for one hour, its pH adjusted to 6 with concentrated NaOH and the material fed immediately to patients with pernicious anemia.

<sup>1</sup> All surgical procedures were carried out under full ether anesthesia.

<sup>2</sup> Supplied through the courtesy of Vegex, Inc., New York City.

The livers of achlorhydric, anemic swine, as well as of normal controls, were extracted in order to test their anti-pernicious-anemia effect. The method of extraction was that described by Cohn, McMeekin and Minot (18). The fresh liver was ground into water and the resulting suspension was brought to a concentration of 70 per cent alcohol. The filtrate was then concentrated in vacuo and brought to a concentration of 95 per cent alcohol by the addition of a suitable amount of absolute alcohol. The resultant precipitate was dissolved in water, neutralized, filtered and sterilized by boiling to prepare it for injection.

The content of vitamin B in the Goldberger diet used in these experiments was determined by testing its power to promote growth in young rats. A standard procedure was employed. Animals weighing from 40 to 50 grams were fed immediately after weaning a basal diet deficient in vitamin B. They were fed only this diet for two weeks in order to ensure the complete depletion of the body store of vitamin B<sub>1</sub> and B<sub>2</sub> (G). After that time they were fed only the diet which produces black-tongue. The animals were weighed weekly and the rate of gain in weight was compared with that of rats fed a normal diet at the same season of the year. These latter data were obtained from the report of Levene (19).

## RESULTS

A certain number of swine died suddenly, either before or shortly after blood changes had occurred. Some of these deaths were due to acute infections, while others could not be explained at post-mortem examination. Such animals are not included in the experimental results.

In all the surviving swine a well defined symptom-complex appeared which presented certain similarities to canine black-tongue, but which was

in no instance absolutely identical with that disease. The most striking manifestations were stomatitis, achlorhydria and anemia. Diarrhea, loss of appetite and weakness also occurred with fair regularity. On the basis of the type of the anemia, two distinct types of reaction were observed. In one, there was an increase in the average red cell diameter and in the hemoglobin content of each individual erythrocyte; and in the other, the reverse was true, namely, the average red blood cell was smaller and its hemoglobin content was less than normal. Accordingly, one group was designated as that with macrocytic anemia, and the other as the microcytic-anemia group.

The control animals on the basal diet failed to develop either the symptoms or the hematological changes which were so constant in the swine fed the diet producing black-tongue. Furthermore, the pathological changes of the bone marrow were wholly dissimilar.

## SYMPTOMATOLOGY

A tabulation of the symptoms as they occurred in the swine of both the macrocytic- and microcytic-anemia groups is presented in Table I. With the exception of diarrhea, the disease manifestations other than hematological occurred more frequently in the group of animals which developed the macrocytic type of anemia. There was a striking tendency to remission and exacerbation of symptoms.

TABLE I  
*The incidence and time of appearance of symptoms in the three experimental groups of swine*

Group I macrocytic anemia							Group II microcytic anemia							Control swine			
Swine number	Duration	Mouth lesions	Diarrhea	Paralysis	Time of appearance of achlorhydria	Time of appearance of anemia	Swine number	Duration	Mouth lesions	Diarrhea	Paralysis	Time of appearance of achlorhydria	Time of appearance of anemia	Swine number	Duration	Time of appearance of achlorhydria	Time of appearance of anemia
1	days				weeks	weeks	10	days				weeks	weeks		days	weeks	weeks
2	74	+	+	0	7	8	11	51	0	++	0	5	5	16	145	2	0
3	83	0	++	0		8	12	85	0	++	0	6	5	17	109	3	0
4	111	+	++	+	7	7	13	42	0	++	0	5	5	18	89	3	0
5	93	++	++	++	11	6	14	74	0	++	0	4	4	19	91	3	0
6	132	0	++	0	4	11	15	65	+	++	+	7	7				
7	127	0	++	+	16	4		120	0	+	+	6	10				
8	47	+	++	0		6											
9	82	+	++	0	7	4											
	121	+	++	0	6	6											
Average	98							73							104		

has been described by Cowgill and Gilman (13). Changes in the central nervous systems of dogs fed diets deficient in vitamin B<sub>2</sub> (G) rather than in anti-neuritic vitamin have been published by Zimmerman and Burack (14). Since the diet used in the experiments reported in this communication produced canine black-tongue, but was found to contain both of these essential food substances when tested on the rat, it is difficult to evaluate the pertinence of those previous studies in which there was an almost complete lack of the vitamin B fractions.

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In order to ascertain the effect of a deficiency of vitamins, other than of the water-soluble vitamin B complex, control experiments were performed. Swine similar to those used in the major experiment were kept under similar conditions and were fed a diet which was vitamin deficient, but was otherwise adequate. By adding suitable supplementary materials this diet could be made deficient in any single vitamin or combination of vitamins which it was desired to study. The basal diet used was the one recommended by Cowgill and Gilman (13) and consisted of the ingredients shown in Table B.

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Water .....	12.2 grams

from the femoral vein. The erythrocytes were counted in the usual manner, using pipettes certified for accuracy by the U. S. Bureau of Standards. The hemoglobin determinations were made by the Sahli acid hematin method, using calibrated tubes and standards which were frequently checked by the Van Slyke (15) method of determining O<sub>2</sub> capacity. The mean corpuscular volume was calculated by the hematocrit method of Wintrobe (16). Reticulocytes were stained by allowing the wet blood smear to come in contact with dry brilliant cresyl blue stain. They were later counterstained by Wright's method.

Studies of the gastric secretion were made on all the animals at two-week intervals. Only two samples were examined, one obtained from the fasting stomach and a second withdrawn from 20 to 30 minutes after the intramuscular injection of histamine. These specimens were titrated in the usual way with 0.1 normal NaOH, using Töpfer's reagent and phenolphthalein as indicators. Control studies of the histology of the bone marrow of the experimental animals were made on tissue removed at operation from the femur.<sup>1</sup>

All of the swine were autopsied, at which time specimens of various tissues including the bone marrow were fixed in Zenker's fluid containing 5 per cent acetic acid as well as in a 10 per cent solution of formaldehyde in Zenker's fluid. The tissues fixed in the Zenker's fluid with acetic acid were stained with eosin and methylene blue. Material fixed by other fluids was stained by the Giemsa method as modified by Wolbach (17).

Gastric juice in amounts sufficient to allow it to be tested for its content of anti-pernicious-anemia substance was obtained from individual swine which had been allowed no food for twenty-four hours. A stomach tube was inserted and the fasting gastric content was removed and discarded. One cubic centimeter of a 1:1000 dilution of histamine was then injected intramuscularly and all the fluid secreted in response to that stimulus was collected. Great care was exercised to keep the gastric juice cold and strongly acid until it was used.

In preparing the mixtures of gastric juice and yeast extract, the juice was first filtered through fine gauze. Twelve grams of yeast extract (Vegex<sup>2</sup>) were then suspended in each 150 cc. of the fluid. The mixture was incubated at 37.5° for one hour, its pH adjusted to 6 with concentrated NaOH and the material fed immediately to patients with pernicious anemia.

<sup>1</sup> All surgical procedures were carried out under full ether anesthesia.

<sup>2</sup> Supplied through the courtesy of Vegex, Inc., New York City.

The livers of achlorhydric, anemic swine, as well as of normal controls, were extracted in order to test their anti-pernicious-anemia effect. The method of extraction was that described by Cohn, McMeekin and Minot (18). The fresh liver was ground into water and the resulting suspension was brought to a concentration of 70 per cent alcohol. The filtrate was then concentrated in vacuo and brought to a concentration of 95 per cent alcohol by the addition of a suitable amount of absolute alcohol. The resultant precipitate was dissolved in water, neutralized, filtered and sterilized by boiling to prepare it for injection.

The content of vitamin B in the Goldberger diet used in these experiments was determined by testing its power to promote growth in young rats. A standard procedure was employed. Animals weighing from 40 to 50 grams were fed immediately after weaning a basal diet deficient in vitamin B. They were fed only this diet for two weeks in order to ensure the complete depletion of the body store of vitamin B<sub>1</sub> and B<sub>2</sub> (G). After that time they were fed only the diet which produces black-tongue. The animals were weighed weekly and the rate of gain in weight was compared with that of rats fed a normal diet at the same season of the year. These latter data were obtained from the report of Levene (19).

## RESULTS

A certain number of swine died suddenly, either before or shortly after blood changes had occurred. Some of these deaths were due to acute infections, while others could not be explained at post-mortem examination. Such animals are not included in the experimental results.

In all the surviving swine a well defined symptom-complex appeared which presented certain similarities to canine black-tongue, but which was

in no instance absolutely identical with that disease. The most striking manifestations were stomatitis, achlorhydria and anemia. Diarrhea, loss of appetite and weakness also occurred with fair regularity. On the basis of the type of the anemia, two distinct types of reaction were observed. In one, there was an increase in the average red cell diameter and in the hemoglobin content of each individual erythrocyte; and in the other, the reverse was true, namely, the average red blood cell was smaller and its hemoglobin content was less than normal. Accordingly, one group was designated as that with macrocytic anemia, and the other as the microcytic-anemia group.

The control animals on the basal diet failed to develop either the symptoms or the hematological changes which were so constant in the swine fed the diet producing black-tongue. Furthermore, the pathological changes of the bone marrow were wholly dissimilar.

## SYMPTOMATOLOGY

A tabulation of the symptoms as they occurred in the swine of both the macrocytic- and microcytic-anemia groups is presented in Table I. With the exception of diarrhea, the disease manifestations other than hematological occurred more frequently in the group of animals which developed the macrocytic type of anemia. There was a striking tendency to remission and exacerbation of symptoms.

TABLE I  
*The incidence and time of appearance of symptoms in the three experimental groups of swine*

Group I macrocytic anemia							Group II microcytic anemia							Control swine			
Swine number	Duration	Mouth lesions	Diarrhea	Paralysis	Time of appearance of achlorhydria	Time of appearance of anemia	Swine number	Duration	Mouth lesions	Diarrhea	Paralysis	Time of appearance of achlorhydria	Time of appearance of anemia	Swine number	Duration	Time of appearance of achlorhydria	Time of appearance of anemia
	days				weeks	weeks		days				weeks	weeks		days	weeks	weeks
1	74	+	+	0	7	8	10	51	0	+	0	5	5	16	145	2	0
2	83	0	+	0		8	11	85	0	+	0	6	7	17	100	3	0
3	111	+	+	+	7	7	12	42	0	+	0	5	5	18	80	3	0
4	93	+	+	+	11	6	13	74	0	+	0	4	4	19	91	3	0
5	132	0	+	0	4	11	14	65	+	+	0		7				
6	127	0	+	+	16	4	15	120	0	+	+	6	10				
7	47	+	+	+		6											
8	82	0	+	0	7	4											
9	121	+	+	0	6	6											
Average	98						73							104			



*Oral lesions.* Of the nine animals in the first, or macrocytic-anemia group, five had lesions of the lingual, buccal or pharyngeal mucous membrane. Only one animal of the six included in the second, or microcytic-anemia group, showed similar changes. The most common lesion was a circumscribed ulceration, most commonly of the lower lip or tongue, showing a red indurated border and base, and a yellow necrotic center. There was little or no localized injection about these ulcers. In the early stages the lesions were usually very small, measuring 0.2 mm. to 0.5 mm. in diameter. As they progressed, they coalesced to form large necrotic areas which were occasionally 1 to 2 cm. in diameter. At that stage the ulcers were surrounded by faintly injected tissue, the margins were elevated, and the centers sharply depressed. The lesions healed slowly and often left residual scars. The time of appearance of ulceration varied considerably; in several of the animals it appeared in the fifth or sixth week after the experimental feeding was begun, while in others it occurred much later. There seemed to be a definite relation between the appearance of these lesions and the development of other symptoms, and in certain instances recurrent ulceration was a feature.

Glossitis was seen in only two animals, both of which were in the first group. The earliest manifestation was redness of the tongue along the borders and over the tip. In a few days atrophy of the papillae in those regions developed, leaving a smooth glossy surface. After a brief interval the redness faded, and the papillae regenerated. The changes were very similar to those described in chronic canine black-tongue.

*Gastro-intestinal disturbances.* The animals ate well until the onset of severe mouth lesions, when the food was taken reluctantly and at times refused. The majority of the animals did not lose weight until late in the disease. Diarrhea occurred in all of the animals of both groups. The stools were voluminous, soft and yellow in color. The animals, although not full-grown, failed to gain weight after the first few weeks following the institution of the diet.

*Disturbance of motor function.* Three of the animals which developed macrocytic anemia, and one animal showing microcytic anemia, developed

striking motor weakness of the limbs during the course of the disease. The earliest manifestation was unsteadiness of the gait, which progressed until there was an apparently complete loss of motor function of the extremities. The forelegs were the last to be involved. Treatment with intramuscularly injected liver extract was effective in relieving the weakness, whereas no effect was observed following oral treatment with large amounts of a potent vitamin B<sub>1</sub> concentrate.<sup>3</sup> Hence, it appears that this symptom was not due to a lack of the anti-neuritic vitamin.

### *Achlorhydria*

Control gastric analyses done at the beginning of the experimental feeding demonstrated the presence of free hydrochloric acid in the gastric juice of all the animals, either in the fasting contents or in the specimen obtained after the injection of histamine. In Table I are presented for all the animals the time of disappearance of free hydrochloric acid from the gastric juice after the injection of histamine. Seven of the animals of the first group developed a complete achlorhydria during the experimental feeding. In every instance except one the achlorhydria was persistent. The time at which it appeared was variable. Four of the animals of the second, or microcytic-anemia group, developed a similar achlorhydria, a change which preceded the appearance of anemia in every instance.

### *Anemia*

Anemia occurred in all of the swine which survived more than two months. The classification of the anemia into two groups, according to the size and hemoglobin content of the erythrocytes, has been discussed. Nine of the fifteen animals presented developed a macrocytic anemia, and six developed the microcytic type.

#### GROUP I. MACROCYTIC ANEMIA

The maximum variation in blood values observed in the nine animals of this group, together with their original blood levels, are presented in Table II. In each instance the mean corpuscular volume and the color index were higher at the low point of the anemia than at the commence-

<sup>3</sup> Rice polishings concentrate furnished through the courtesy of the Burroughs Wellcome Company, Tuckahoe, New York.

TABLE II  
The maximum variations in blood levels in the group of swine with macrocytic anemia.  
Group I. Macrocytic anemia

Swine number	Blood studies before experimental diet					Blood studies at low point of anemia				
	R.B.C.	Hgb.†	W.B.C.	MCV*	CI*	R.B.C.	Hgb.†	W.B.C.	MCV	CI
	millions	per cent		per cent		millions	per cent		per cent	
1	8.69	84	19,150	40.5	.485	4.30	52	20,850	62.0	.605
2	7.58	85	23,850	55.0	.530	4.89	53	5,600	48.0	.550
3	8.13	80	13,700	46.0	.495	3.55	41	22,100	63.0	.585
4	8.26	88	18,500	48.5	.535	3.17	48	19,600	61.0	.775
5	8.43	80	22,600	49.0	.475	2.25	31	14,900	62.5	.705
6	7.14	79	22,900	52.0	.555	4.48	53	19,100	63.0	.605
7	6.18	80	18,600	51.0	.655	2.80	44	1,900	52.5	.785
8	7.54	75	11,400	47.0	.500	4.04	45	16,900	58.0	.715
9	7.73	89	24,000	45.0	.575	3.50	50	34,900	65.5	.625
Average	7.74	82		48.2	.534	3.66	46.3		59.5	.653
Per cent change						-53	-43.5		+23.5	+22.4

\* MCV = mean corpuscular volume.

CI = color index.

† 100 per cent Hgb. = 20.6 volumes per cent O<sub>2</sub> capacity.

ment of the experimental feeding, changes in keeping with the fact that the red blood cell count showed a greater proportionate decrease than did the hemoglobin level.

At the outset the average erythrocyte count of all the animals of this group was 7,740,000, and the average hemoglobin level was 82 per cent. At the same time, the average mean corpuscular volume was 48.2 cubic microns, and the average color index was .534. At the low point of the anemia, the average erythrocyte count of all the animals was 3,660,000, or a decrease of 53 per cent. The average hemoglobin level was 46.3 per cent, or a decrease of 43.5 per cent. At the same time, there occurred an increase in the average mean corpuscular volume from 48.2 to 59.5 cubic microns, or an increase of 23.5 per cent. The average color index increased from .534 to .653, or an increase of 22.4 per cent. The disproportion between the number of erythrocytes and hemoglobin levels was reflected in the increase in the size of the average red blood cell and in the increase in its hemoglobin content.

#### PROTOCOLS OF TYPICAL EXPERIMENTS

##### Swine number 2, Figure 1

1933

June 5 Weight 13.3 kgm. Feeding of experimental diet begun.

June 12 R.B.C. 7.73 Hgb. 89% MCV 45.0 CI .575  
W.B.C. 24,800

June 20	Gastric analysis:	Vol.	Free HCl	Total acid.
	Fasting	30 cc.	10.0	37.5
	30' p Histamine	30 cc.	36.5	56.3
June 27	Weight 13.9 kgm. No symptoms.			
July 5	Diarrhea has been present for four days. R.B.C. 5.31 Hgb. 52% MCV 48.0 CI .490			
July 13	Gastric analysis:	Vol.	Free HCl	Total acid.
	Fasting	15 cc.	0	14.5
	30' p Histamine	19 cc.	0	27.5
	Ten cc. of a rice polishings concentrate by mouth has been administered daily for two days. The diarrhea has ceased.			
Aug. 10	Iron ammonium citrate 1 gram daily has been added to the diet.			
Aug. 15	The animal has been symptomatically well and now has a severe anemia. R.B.C. 3.86 Hgb. 34% MCV 68.0 CI .700 Weight 12.9 kgm.			
Aug. 29	The anemia persists but the animal appears to be well otherwise.			
Sept. 10	Weight 13.9 kgm. No evidence of disease except for anemia.			
Sept. 16	Diet refused. Diarrhea recurred. A concentrate of rice polishings was administered in 10 cc. amounts daily by mouth. R.B.C. 3.50 Hgb. 50% MCV 65.5 CI .715			
Sept. 20	Gastric analysis:	Vol.	Free HCl	Total acid.
	Fasting	21 cc.	16.6	22.2
	30' p Histamine	30 cc.	59.5	73.6
	Free HCl is now present in the gastric juice and some improvement in blood values has occurred. R.B.C. 4.64 Hgb. 51% MCV 65.5 CI .755			



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Anemia occurred in all of the swine which survived more than two months. The classification of the anemia into two groups, according to the size and hemoglobin content of the erythrocytes, has been discussed. Nine of the fifteen animals presented developed a macrocytic anemia, and six developed the microcytic type.

#### GROUP I. MACROCYTIC ANEMIA

The maximum variation in blood values observed in the nine animals of this group, together with their original blood levels, are presented in Table II. In each instance the mean corpuscular volume and the color index were higher at the low point of the anemia than at the commence-

<sup>3</sup> Rice polishings concentrate furnished through the courtesy of the Burroughs Wellcome Company, Tuckahoe, New York.

TABLE II

*The maximum variations in blood levels in the group of swine with macrocytic anemia.  
Group I. Macrocytic anemia*

Swine number	Blood studies before experimental diet					Blood studies at low point of anemia				
	R.B.C.	Hgb.†	W.B.C.	MCV*	CI*	R.B.C.	Hgb.†	W.B.C.	MCV	CI
	millions	per cent		per cent		millions	per cent		per cent	
1	8.69	84	19,150	40.5	.485	4.30	52	20,850	62.0	.605
2	7.58	85	23,850	55.0	.530	4.89	53	5,600	48.0	.550
3	8.13	80	13,700	46.0	.495	3.55	41	22,100	63.0	.585
4	8.26	88	18,500	48.5	.535	3.17	48	19,600	61.0	.775
5	8.43	80	22,600	49.0	.475	2.25	31	14,900	62.5	.705
6	7.14	79	22,900	52.0	.555	4.48	53	19,100	63.0	.605
7	6.18	80	18,600	51.0	.655	2.80	44	1,900	52.5	.785
8	7.54	75	11,400	47.0	.500	4.04	45	16,900	58.0	.715
9	7.73	89	24,000	45.0	.575	3.50	50	34,900	65.5	.625
Average	7.74	82		48.2	.534	3.66	46.3		59.5	.653
Per cent change						-53	-43.5		+23.5	+22.4

\* MCV = mean corpuscular volume.

CI = color index.

† 100 per cent Hgb. = 20.6 volumes per cent O<sub>2</sub> capacity.

ment of the experimental feeding, changes in keeping with the fact that the red blood cell count showed a greater proportionate decrease than did the hemoglobin level.

At the outset the average erythrocyte count of all the animals of this group was 7,740,000, and the average hemoglobin level was 82 per cent. At the same time, the average mean corpuscular volume was 48.2 cubic microns, and the average color index was .534. At the low point of the anemia, the average erythrocyte count of all the animals was 3,660,000, or a decrease of 53 per cent. The average hemoglobin level was 46.3 per cent, or a decrease of 43.5 per cent. At the same time, there occurred an increase in the average mean corpuscular volume from 48.2 to 59.5 cubic microns, or an increase of 23.5 per cent. The average color index increased from .534 to .653, or an increase of 22.4 per cent. The disproportion between the number of erythrocytes and hemoglobin levels was reflected in the increase in the size of the average red blood cell and in the increase in its hemoglobin content.

#### PROTOCOLS OF TYPICAL EXPERIMENTS

##### Swine number 2, Figure 1

1933

June 5 Weight 13.3 kgm. Feeding of experimental diet begun.

June 12 R.B.C. 7.73 Hgb. 89% MCV 45.0 CI .575  
W.B.C. 24,800

June 20	Gastric analysis:	Vol.	Free HCl	Total acid.
	Fasting	30 cc.	10.0	37.5
	30' p Histamine	30 cc.	36.5	56.3
June 27	Weight 13.9 kgm.	No symptoms.		
July 5	Diarrhea has been present for four days.			
	R.B.C. 5.31 Hgb. 52% MCV 48.0 CI .490			
July 13	Gastric analysis:	Vol.	Free HCl	Total acid.
	Fasting	15 cc.	0	14.5
	30' p Histamine	19 cc.	0	27.5
	Ten cc. of a rice polishings concentrate by mouth has been administered daily for two days. The diarrhea has ceased.			
Aug. 10	Iron ammonium citrate 1 gram daily has been added to the diet.			
Aug. 15	The animal has been symptomatically well and now has a severe anemia.			
	R.B.C. 3.86 Hgb. 34% MCV 68.0 CI .700			
	Weight 12.9 kgm.			
Aug. 29	The anemia persists but the animal appears to be well otherwise.			
Sept. 10	Weight 13.9 kgm. No evidence of disease except for anemia.			
Sept. 16	Diet refused. Diarrhea recurred. A concentrate of rice polishings was administered in 10 cc. amounts daily by mouth.			
	R.B.C. 3.50 Hgb. 50% MCV 65.5 CI .715			
Sept. 20	Gastric analysis:	Vol.	Free HCl	Total acid.
	Fasting	21 cc.	16.6	22.2
	30' p Histamine	30 cc.	59.5	73.6
	Free HCl is now present in the gastric juice and some improvement in blood values has occurred.			
	R.B.C. 4.64 Hgb. 51% MCV 65.5 CI .555			

- Sept. 27 The animal is eating poorly and has diarrhea. R.B.C. 4.82 Hgb. 60% MCV 55.0 CI .625
- Oct. 3 There are several small circumscribed ulcers of the tongue, lower lip, and pharynx. The appetite is poor and the animal appears to be ill.  
R.B.C. 4.01 Hgb. 60% W.B.C. 1,600  
MCV 56.0 CI .750
- Oct. 4 Found dead. Autopsy: There were deep ulcerated lesions of lips, tongue, and pharynx. The marrow was deep red and appeared to be hyperplastic.

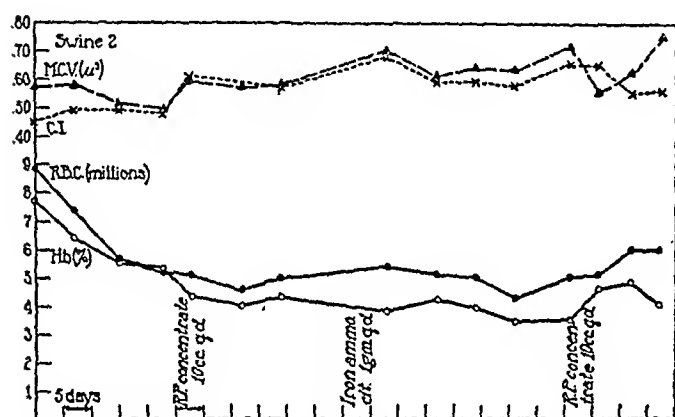


FIG. 1. GRAPH SHOWING THE CHANGES IN BLOOD LEVELS OF SWINE 2.

In this animal (Figure 1) recurrent mouth lesions, diarrhea, anemia and achlorhydria were present for a period of 121 days. The anemia was persistent and was accompanied by an increase in the size of the red blood cell and its hemoglobin content. No detectable effect followed the administration of iron by mouth. A rice polishings concentrate which contained a relatively large amount of vitamin B<sub>1</sub> and very little vitamin B<sub>2</sub> (G) was given during the period of achlorhydria, and no immediate improvement in the blood values was observed. How-

ever, later in the experimental period free HCl reappeared in the gastric juice, the same concentrate of rice polishings was given and a blood remission ensued. The significance of this observation is not clear.

#### Swine number 3 (Figure 2)

1934

- March 1 Feeding of experimental diet begun.  
R.B.C. 8.13 Hgb. 80% MCV 46.0 CI .495
- March 7 Weight 11.8 kgm.
- | Gastric analysis: | Vol.   | Free acid. | Total acid. |
|-------------------|--------|------------|-------------|
| Fasting           | 20 cc. | 28         | 36          |
- March 24 The animal has been well and has gained weight.  
Weight 13.0 kgm.
- April 4 R.B.C. 5.15 Hgb. 52% MCV 47.0 CI .510  
No evidence of disease is present.
- April 15 The diet is taken poorly. There is smoothness, atrophy, and moderate redness of the tongue. Several small circumscribed punched-out ulcers are present on the buccal mucosa. The legs are weak.
- April 21 R.B.C. 3.77 Hgb. 36% MCV 45.0 CI .510  
Diarrhea has been present for 4 or 5 days. The appetite is poor and the mouth lesions persist. In spite of marked weakness all the limbs can be moved. The daily intramuscular administration of liver extract in 5 cc. amounts was begun today. Reticulocyte count 0.6 per cent.
- April 26 There are a very few deep circumscribed necrotic lesions of tongue and labial mucosa. Diarrhea has ceased.  
R.B.C. 3.55 Hgb. 41% MCV 63.0 CI .585  
Reticulocyte count 7.2 per cent.
- April 28 Apparently complete motor weakness of the limbs is present. Mouth rapidly improving. No diarrhea. Eating well.  
Reticulocyte count 11.2 per cent.

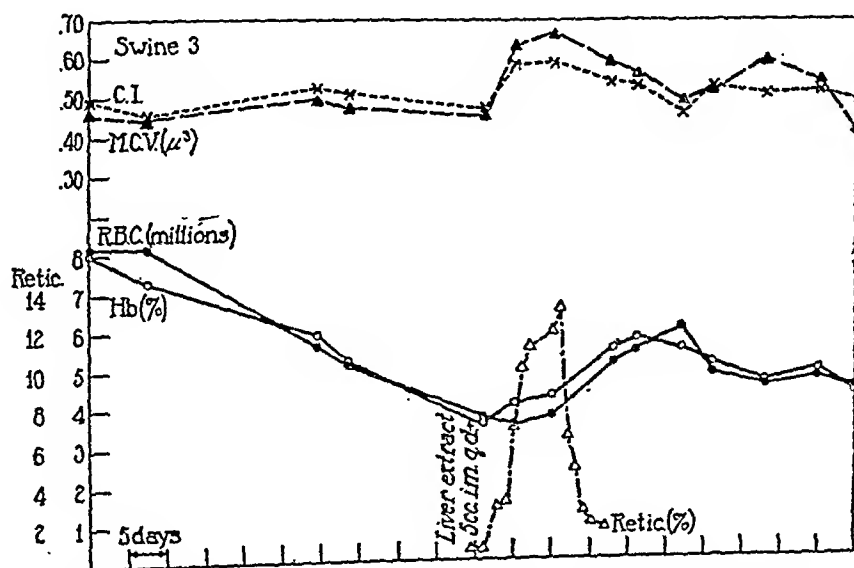


FIG. 2. GRAPH SHOWING CHANGES IN BLOOD LEVELS OF SWINE 3.

April 30	Now regaining the function of hind legs. Mouth nearly healed.			
May 1	R.B.C. 3.78 Hgb. 43% MCV 66.0 CI .590 Reticulocytes 12 per cent.			
May 4	Animal in good condition. Eating well and is up and about. Mouth healed. Daily injections of liver extract discontinued.			
May 5	Gastric analysis:	Vol.	Free HCl	Total acid.
	Fasting	11 cc.	0	4.7
	30' p Histamine	30 cc.	0	16.9
May 12	R.B.C. 5.45 Hgb. 58% MCV 56.0 CI .536 The animal is now in very good condition.			
May 19	Refused food today. Has frequent voluminous yellow stools. R.B.C. 6.04 Hgb. 55% MCV 49.0 CI .460			
May 24	Liver extract in ten gram amounts has been given daily by mouth for four days. The animal is now symptom-free. There is no diarrhea and the diet is taken well.			

This animal (Figure 2) developed recurrent mouth lesions, a macrocytic anemia and achlorhydria. It survived for 111 days. The anemia was relieved by the daily intramuscular injection of 2.5 cc. of liver extract (Lilly number 343). A reticulocyte rise of 13.2 per cent occurred on the seventh day after the beginning of therapy, and was followed by improvement of the blood levels. The red blood cell count rose from 3,550,000 with a hemoglobin of 41 per cent to a count of 6,040,000 and a hemoglobin of 55 per cent. There was a marked degree of weakness of the extremities which disappeared after the intramuscular injection of liver extract.

#### GROUP II. MICROCYTIC ANEMIA

The maximum alterations of the blood levels observed in the six animals of this group, together with the original levels, are presented in Table III. In five of the swine the mean corpuscular volume of the red blood cells was lower during

the anemia than were the original values. The sixth animal, Number 15, is included in this group because the color index was not increased, although the mean corpuscular volume did not fall below the normal level. While none of these animals showed an anemia which was markedly microcytic in character, yet the contrast to the type of cell seen in the macrocytic group was striking.

The average original red blood cell count of all the animals of this group was 6,790,000, and the average hemoglobin level was 76.9 per cent. The average mean corpuscular volume was 60.6 cubic microns, while the average color index was .570. At the low point of the anemia the average erythrocyte count was 3,990,000, a decrease of 41 per cent. At the same time, the average hemoglobin level had fallen to 44.6 per cent, or a decrease of 42.5 per cent. The average mean corpuscular volume fell to 48.5 cubic microns, or a decrease of 4.5 per cent, while the color index decreased to 0.556, or a decrease of 2.25 per cent. It is apparent that the average decrease of the numbers of red blood cells was less than the average decrease of the hemoglobin values. Accompanying this anemia, the average red blood cell became smaller and its hemoglobin content was diminished in comparison with the original values.

#### PROTOCOLS

##### Swine number 13 (Figure 3)

1933

Aug. 7 Experimental diet commenced. Weight 9.3 kgm. This animal received the modified Goldberger diet plus reduced iron, one gram daily, throughout the course of the study.

TABLE III  
*The average maximum variations in blood values of the experimental swine with microcytic anemia*  
Group II. Microcytic anemia

Swine number	Blood studies before experimental diet					Blood studies at low point of anemia				
	R.B.C.	Hgb.	W.B.C.	MCV	CI	R.B.C.	Hgb.	W.B.C.	MCV	CI
	millions	per cent		per cent		millions	per cent		per cent	
10	7.09	84	23,500	53.5	.600	3.87	39	6,590	54.0	.515
11	6.11	67	19,300	52.0	.550	4.58	57	7,400	53.5	.540
12	7.41	83	19,000	48.0	.560	3.67	40	35,000	46.5	.555
13	6.00	72	15,900	56.0	.600	2.87	31	3,930	42.5	.555
14	6.69	73	20,000	50.5	.560	4.22	49	8,250	45.0	.545
15	7.49	82	24,700	44.0	.550	4.74	52	12,000	49.5	.560
Average	6.79	76.9		50.6	.570	3.92	44.6		48.5	.556
Per cent of change						-41.0	-42.5		-4.5	-2.25

Aug. 12	Gastric analysis:	Vol.	Free HCl	Total acid.
	Fasting	30 cc.	2.0	4.0
	30' p Histamine	30 cc.	34.2	12.2
	R.B.C. 6.44 Hgb. 96% MCV 58.5 CI .750			
Aug. 25	The animal has been well and has had no symptoms.			
Sept. 13	Gastric analysis:	Vol.	Free HCl	Total acid.
	Fasting	5 cc.	0	9.6
	30' p Histamine	17 cc.	0	14.4
	The animal has eaten well and is symptom-free.			
Sept. 21	R.B.C. 4.46 Hgb. 48% MCV 50.0 CI .540			
	An anemia is now present, together with an achlorhydria. Diarrhea has occurred irregularly during the past week.			
Oct. 9	R.B.C. 3.87 Hgb. 38% MCV 46.5 CI .495			
	The animal appears well. There is no diarrhea, but there has been some loss of weight.			
Oct. 13	Gastric analysis:	Vol.	Free HCl	Total acid.
	Fasting	9 cc.	0	5.2
	30' p Histamine	15 cc.	0	8.0
Oct. 17	R.B.C. 3.23 Hgb. 33% MCV 41.5 CI .515			
	Appears weak, has diarrhea, but is eating. The skin and mucous membranes appear yellow and the animal has a microcytic anemia.			
Oct. 19	R.B.C. 2.89 Hgb. 31% MCV 42.5 CI .555			
	Profuse diarrhea, refused food today and appeared very weak. Sudden death occurred. Autopsy: There was an effusion of yellow bile-stained fluid in all the serous cavities. The liver presented an irregular golden brown coloration. The femoral, rib and			

sternal marrows were very red and solid in consistency.

This animal is presented because the blood studies are typical of those seen in the animals of this group. It had a severe microcytic anemia although iron was administered daily during the entire period of the experimental feeding. No mouth lesions were seen. Achlorhydria developed early in the course of the disease and was persistent.

#### Swine number 15 (Figure 4)

1933	
Aug. 7	Feeding of experimental diet begun. The diet was supplemented by one gram daily of reduced iron.
Aug. 14	Weight 12.9 kgm. R.B.C. 7.49 Hgb. 82% MCV 44.0 CI .550
Aug. 22	Gastric analysis: Vol. Free HCl Total acid.
	Fasting 12 cc. 6.0 9.0
	30' p Histamine 18 cc. 17.0 34.0
Aug. 30	R.B.C. 7.09 Hgb. 81% MCV 46.5 CI .580
	The appetite is good. There have been no symptoms.
Sept. 13	Gastric analysis: Vol. Free HCl Total acid.
	Fasting 15 cc. 5.6 18.5
	30' p Histamine 30 cc. 23.2 35.5
	R.B.C. 6.80 Hgb. 71% MCV 44.5 CI .520
Sept. 28	Gastric analysis: Vol. Free HCl Total acid.
	Fasting 11 cc. 0 2.1
	30' p Histamine 30 cc. 0 6.2
	No symptoms have appeared. The animal is well.
Oct. 9	There is definite atrophy of the papillae of the tongue along the borders and over the tip. The animal is not well and is eating reluctantly.
Oct. 17	R.B.C. 5.04 Hgb. 61% MCV 44.0 CI .610
Oct. 23	The animal still appears to be in good condition.
	R.B.C. 5.38 Hgb. 62% MCV 48.5 CI .585
	Gastric analysis: Vol. Free HCl Total acid.
	Fasting 5 cc. 0 6.0
	30' p Histamine 14 cc. 0 12.2
Oct. 28	Weakness of the hind legs and a moderate anemia have appeared. Diarrhea is severe. The oral administration of liver extract, number 343, 4 grams daily, is begun.
	R.B.C. 4.74 Hgb. 52% MCV 50.0 CI .555
Nov. 8	Liver extract treatment is discontinued. The animal is stronger, the weakness of the limbs is no longer present, and an improvement of blood values has occurred.
Nov. 15	R.B.C. 5.22 Hgb. 58% MCV 56.0 CI .565
Nov. 21	R.B.C. 7.11 Hgb. 70% MCV 44.0 CI .490
Dec. 4	After one month of freedom from symptoms

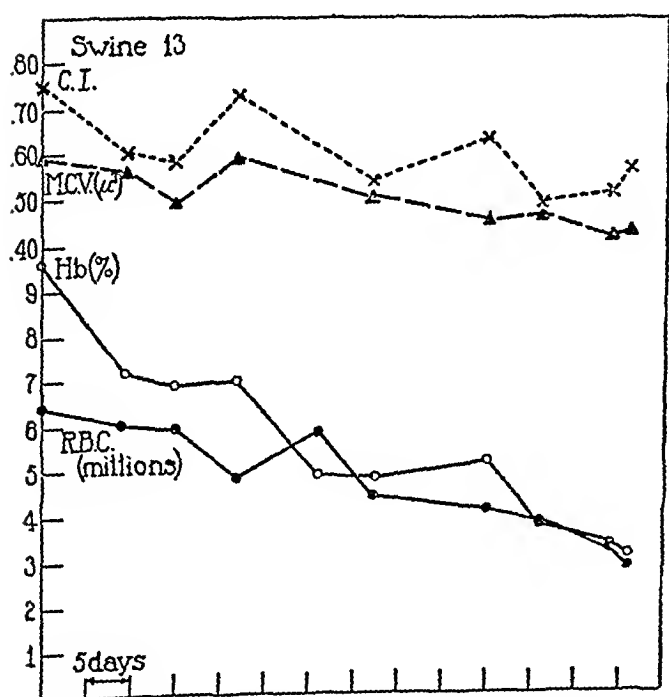


FIG. 3. A GRAPH OF THE CHANGES IN BLOOD LEVELS OF SWINE 13.

the animal appears weak and ill. The diet is refused.

- Dec. 5 Found dead. Autopsy: All the organs appeared normal except for the stomach. The mucosa of the proximal third and of all but the superior surface of the stomach presents a striking alteration. It measures from 2 to 6 mm. in thickness, is a deep brown in color, and is soft and necrotic. There is considerable injection of the submucosa and muscularis. Histological examination shows the mucosa to be necrotic and thickly infiltrated with inflammatory cells and hemorrhagic exudate.

#### CONTROLS

#### *The presence of the vitamin B complex in the experimental diet*

Reference to the literature concerning the diet described by Goldberger as productive of black-tongue in dogs reveals that its content of vitamin B is not clearly understood. In Goldberger's original publications it was assumed that the diet was deficient in a part of the vitamin B complex. This assumption was based upon the fact that the symptoms resulting from the feeding of the diet

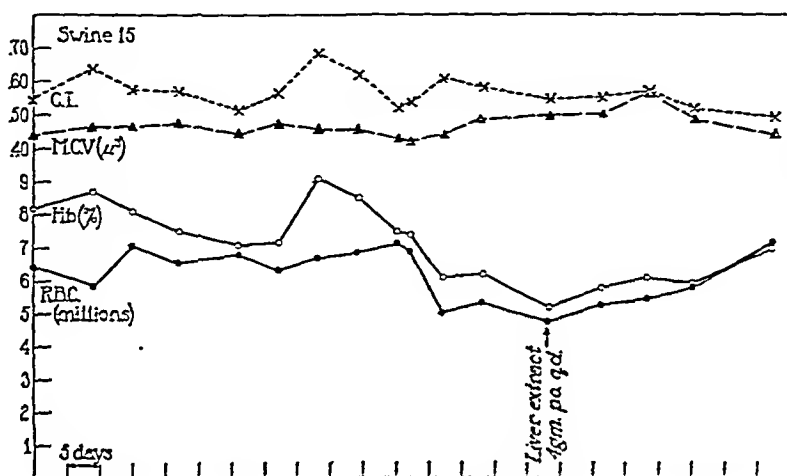


FIG. 4. A GRAPH OF THE CHANGES IN BLOOD LEVELS OF SWINE 15.

In this experiment (Figure 4) the animal presented achlorhydria, anemia which was not macrocytic in character and weakness of the extremities. Atrophy of the papillae of the tongue was intermittently present. The anemia was moderately severe, but the color index and mean corpuscular volume were not altered. There was an improvement of blood values on oral therapy with liver extract.

The protocols are typical of the entire group. The only striking difference between these animals and those previously discussed lies in the fact that the anemia was not markedly macrocytic in type. Iron was given throughout the course of the experiment and did not prevent the anemia. The similarity of the two groups, both in the response to therapy as well as in the pathological alterations of the bone marrow, suggest the conclusion that the two types of anemia are manifestations of a different individual susceptibility.

did not occur if it was supplemented by materials such as yeast, liver, liver extract, meat, milk or a variety of other foodstuffs. The power of preventing black-tongue possessed by those foods was roughly parallel to their known content of water-soluble vitamin B. Hence, the power of preventing black-tongue was supposed to be due to the presence of that vitamin. The dietary constituent which prevented black-tongue was found to be heat-resistant, however, a fact which sharply differentiated it from the thermolabile, anti-neuritic fraction of vitamin B. Accordingly, the fraction which was preventive of black-tongue was named vitamin B<sub>12</sub>, or G. Since it became known that a thermostable, as well as a thermolabile, fraction was necessary for the growth of rats, it was assumed that the factor promoting rat-growth and the one preventing black-tongue were the same.

Experiments were performed to ascertain if the

black-tongue-producing diet, supposedly deficient in thermostable, water-soluble, growth-promoting vitamin, was deficient in fact. This point was of particular importance, since it was found impossible to cause black-tongue by feeding diets devoid of vitamin B and otherwise complete. The technique of the experiments has been described.

necessary to confirm the supposition that a lack of vitamins A, C and D bore no relation to the production of the disease syndrome. The method employed was to feed swine on a basal diet which was known to be deficient in vitamins A, C and D, as well as in  $B_1$  and  $B_2$  (G). To this diet were added substances known to contain the dietary

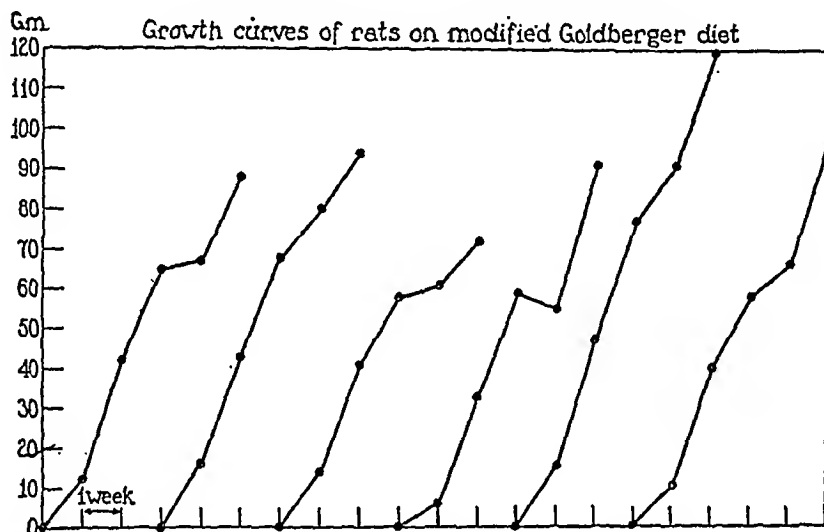


FIG. 5. GRAPH SHOWING THE WEEKLY GAIN IN WEIGHT OF RATS FED THE MODIFIED GOLDBERGER DIET.

In Figure 5 are presented the growth curves of six rats which were fed this modified Goldberger diet over a period of five weeks. All showed a consistent normal weekly growth gain over the entire period, and several gained as much as from 30 to 40 grams per week. The average weekly gain of each animal was 18.7 grams. Therefore, it is evident that the experimental diet which was fed to the swine reported in this communication contained an amount of vitamins  $B_1$  and  $B_2$  (G) adequate to support normal growth in rats. Moreover, similar experiments were performed by feeding the original Goldberger number 123 black-tongue-producing diet without the addition of rice polishings, and exactly the same results were obtained. That diet also contained sufficient vitamin B to allow a normal rate of growth in young rats.

#### *Control for the effect of a deficiency of vitamins other than vitamins $B_1$ and $B_2$ (G)*

Although cod-liver oil was added to the modified Goldberger diet, and although orange juice was given to the pigs receiving this diet, it seemed

anti-anemia constituent. To the diet of two of the animals, 4 grams of Vegex were added, while to the diet of the others 20 grams of egg white were added. Other swine were fed the basal diet without the addition of any material rich in vitamin content.

The animals were kept under the same uniform conditions and studies were made by the same methods and in the same detail as were the studies of the animals fed the modified Goldberger diet. A summary of the hematological studies is presented in Table IV.

Mouth lesions, gastro-intestinal disturbances and paralysis were never observed in any of these animals. All of them became achlorhydric much sooner than did the animals fed the modified Goldberger diet (Table I). This achlorhydria could not be relieved by treating the animals with large amounts of vitamin  $B_1$ . Although the average duration of life was longer for these animals than for any of the others (Table I), no considerable hematological change appeared at any time. On the other hand, similar controls fed the basal vitamin-free diet until death without the addition of substances containing the anti-anemic constituent,

TABLE IV  
The average maximum variation in blood levels of control swine

Swine number	Initial blood levels				Maximum change of blood levels			
	R.B.C.	Hgb.	MCV	CI	R.B.C.	Hgb.	MCV	CI
	<i>millions</i>	<i>per cent</i>	<i>per cent</i>		<i>millions</i>	<i>per cent</i>	<i>per cent</i>	
16	8.14	86	50.0	.530	6.25	70	51.0	.560
17	7.17	69	50.0	.485	7.67	78	43.5	.515
18	8.95	91	41.5	.505	7.10	65	42.0	.454
19	7.44	80	55.5	.540	6.83	76	47.0	.560
Average	7.97	86.5	49.0	.513	6.96	72.5	45.3	.523

also failed to show considerable hematological effects.

To summarize: the modified Goldberger diet contained amounts of vitamin B<sub>1</sub> and B<sub>2</sub> (G) adequate to support a normal rate of growth in rats under standard conditions. Since swine which were fed a diet deficient in vitamins A, C and D, but to which the extrinsic anti-anemia factor was added, did not develop either anemia or other characteristic disease manifestations, it would appear that a lack of vitamins A, C and D bore no relation to the production of the pathological changes under discussion. Furthermore, since the diet which produced the syndrome in animals was shown to contain vitamins B<sub>1</sub> and B<sub>2</sub> (G), and the syndrome could not be produced by feeding diets lacking those vitamins, it would also appear that a deficiency of any known fraction of vitamin B was not etiologic.

#### BONE MARROW STUDIES

*Bone marrow of normal swine.* The femoral marrows of eight normal swine were studied. Grossly, they were very fatty and a bright yellow in color. By microscopic examination (Figure 6) they were extremely hypoplastic with almost the entire marrow space occupied by fat cells. The venous sinusoids were open and occasionally adult red blood cells were seen. In some of the intercellular spaces normoblasts and white blood cells were present.

*Bone marrow of swine with anemia.* There was no appreciable difference detectable in the bone marrows of the two groups of animals. Figure 7 shows a photograph of a biopsy taken from the femur of one of the animals soon after the experimental feeding was begun. This mar-

row is extremely acellular and corresponds in every respect with that of normal animals. In contrast to this is the marrow taken from the same animal at autopsy ten weeks later, when achlorhydria and a severe macrocytic anemia had developed (Figures 8 and 9). The marrow, grossly, was a deep reddish purple in color. Its consistency was firm and elastic. Microscopically, hyperplastic tissue has replaced the fat cells. The blood sinuses are collapsed and adult erythrocytes are rare. The predominating cell is large

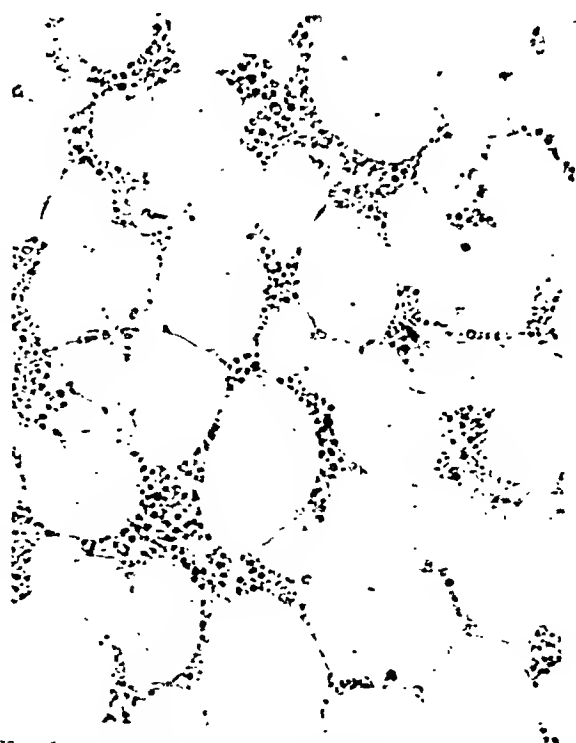


FIG. 6. PHOTOMICROGRAPH OF THE FEMORAL BONE MARROW OF A NORMAL SWINE  
X 300. Giemsa stain.



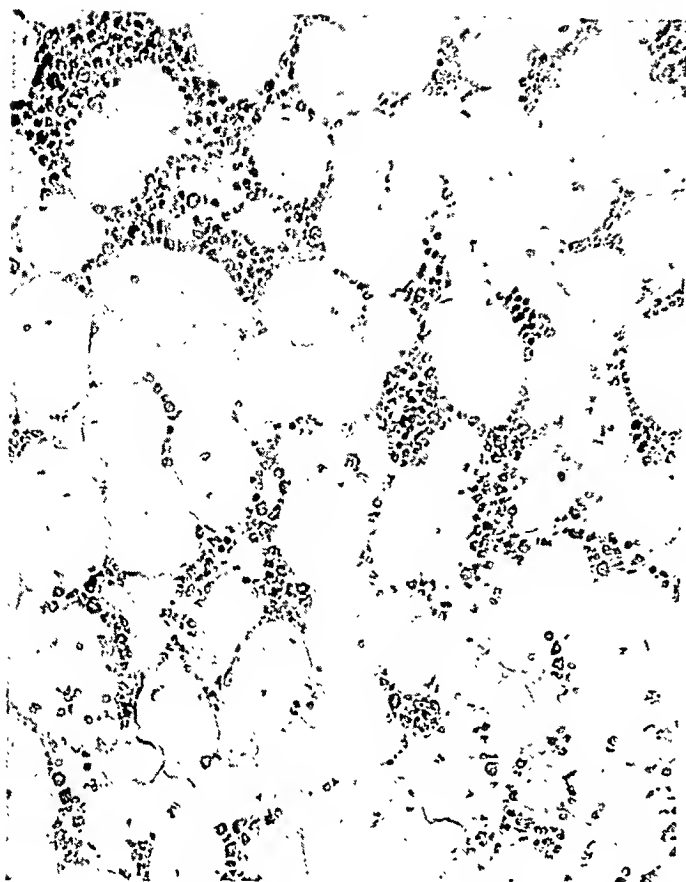


FIG. 7. PHOTOMICROGRAPH OF THE FEMORAL BONE MARROW OF ONE OF THE EXPERIMENTAL SWINE REMOVED BY BIOPSY BEFORE THE DIET FEEDING WAS BEGUN.  $\times 300$ . Giemsa stain.

with a pale, vesicular nucleus, the chromatin of which is arranged loosely about the periphery. The cytoplasm of the cell is basophilic in character and irregular in outline. Mitotic figures are frequent. Classified according to the terminology of Sabin, the cells just described are termed megaloblasts. Erythroblasts, small ovoid cells with heavy nuclear chromatin and hemoglobin in the cytoplasm, both of the early and late types, are more commonly seen than are normoblasts. There is some white-cell hyperplasia, with myelocytes predominating.

Very advanced bone-marrow alterations occurred in only three instances. However, in the remaining marrows varying degrees of cellular hyperplasia of the same general type were present. In many, collections of megaloblasts were found, although the fat spaces had not entirely disappeared. In such marrows late erythroblasts predominate and adult erythrocytes were numerous. In no instance was a marrow of the normoblastic type seen in the microcytic anemias observed. In occasional instances, however, the

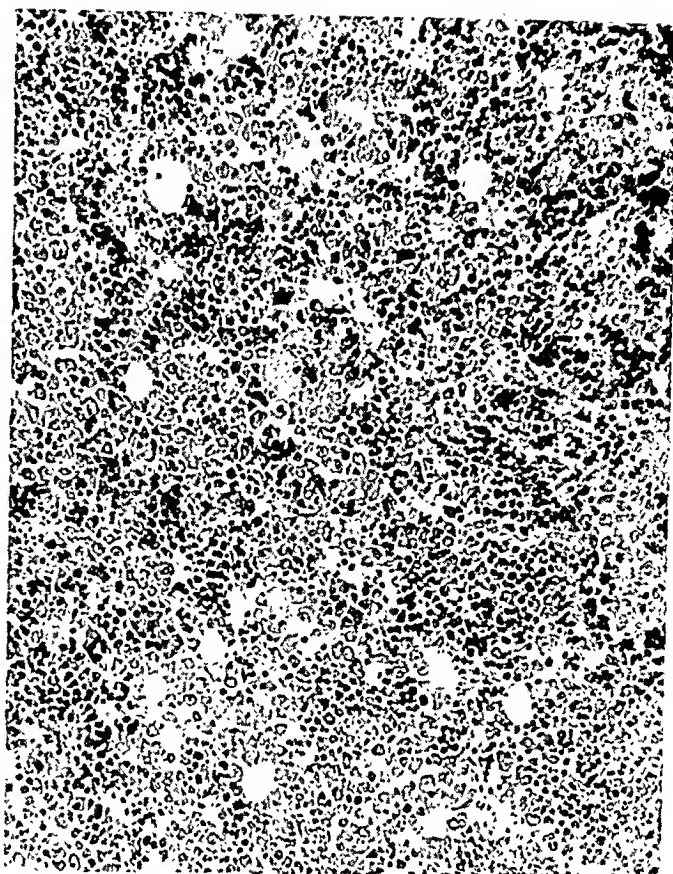


FIG. 8. LOW-POWER PHOTOMICROGRAPH OF THE FEMORAL BONE MARROW OF THE SAME ANIMAL AS IN FIGURE 7, AFTER SEVERE MACROCYTIC ANEMIA HAD DEVELOPED.  $\times 300$ . Giemsa stain.

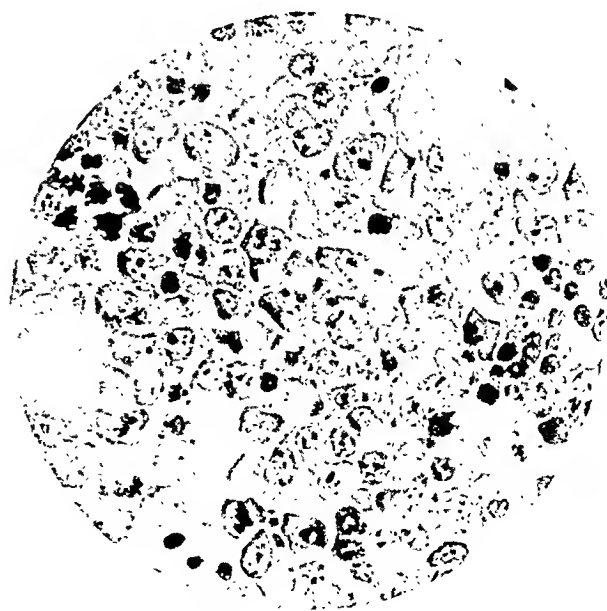


FIG. 9. HIGH-POWER PHOTOMICROGRAPH OF THE SAME FEMORAL MARROW AS SHOWN IN FIGURE 8.  $\times 1000$ . Giemsa stain.

myelocytic hyperplasia was equal to or greater than that of the erythrocytic type. Two of the marrows were not more active than normal. The changes described are in general comparable to those found in the marrow in cases of tropical sprue with anemia, by Rhoads and Castle (20).

*The presence of hematopoietic factor in the gastric juice of swine with experimental achlorhydria and anemia*

The method chosen to determine the presence or absence of the anti-anemic factor in the gastric juice of swine presenting the symptom-complex under discussion was that used by Castle (21) to demonstrate the presence of the same factor in human beings. A digest prepared by incubating 12 grams of Vegex with 150 cc. of gastric juice, obtained from individual swine with achlorhydria and anemia, was fed daily to a patient with pernicious anemia at a low hematological level. After ten days of such treatment gastric juice from normal swine was substituted for the secretion

days a digest with gastric juice from anemic swine was fed, no increase of reticulocytes occurred, and the blood values fell to 1,410,000 erythrocytes and a hemoglobin of 33 per cent. On the tenth day the second period was begun, in which the effect of the substitution of gastric secretion from normal swine for that of the pathological animal was tested. At the beginning of this period the red blood cell count was 1,460,000, the hemoglobin 34 per cent, and the reticulocyte count 0.2 per cent. On the fifth day thereafter, the reticulocyte count rose to 2.0 per cent, on the sixth day to 8.4 per cent, and on the eighth day to 28.2 per cent. This was followed by a rise of the red blood cell count to 1,730,000 and the hemoglobin to 38 per cent on the tenth day, and to 2,060,000 with a hemoglobin of 44 per cent on the fourteenth day. From this experiment it appears that the gastric juice of the swine in which anemia and achlorhydria had developed did not contain the anti-anemia ferment which is present in the gastric juice of normal swine.

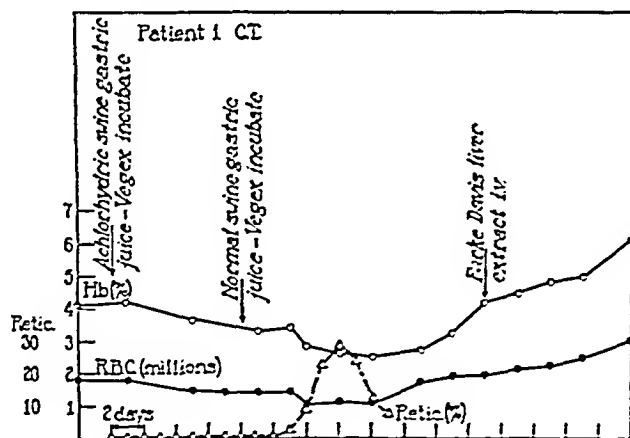


FIG. 10. A GRAPH OF THE CHANGES IN BLOOD LEVELS OF A PATIENT WITH PERNICIOUS ANEMIA DURING THE FEEDING OF DIGESTS OF VEGEX WITH NORMAL AND WITH EXPERIMENTAL SWINE GASTRIC JUICE.

of the pathological animal. The reticulocytes were counted daily.

In Figure 10 and Table V, are presented the blood changes observed in Patient 1 during these two courses of therapy. At the beginning of the experiment the red blood cell count was 1,750,000, the hemoglobin 42 per cent, and the reticulocyte count 0.8 per cent. During the following nine

*Tests for the presence of the anti-anemia factor in the livers of swine with anemia and achlorhydria*

In order to determine the presence of the anti-anemia factor in animals which developed the symptom-complex described, their livers were extracted and the resultant material administered parenterally to patients with pernicious anemia

TABLE V

The changes in blood levels of Patient 1, C.T., with pernicious anemia during the feeding of digests of Vegex with normal and with experimental swine gastric juice

Date	R.B.C.	Hgb.	Therapy	Reticulocytes
1938	millions	per cent		per cent
May 17.....	1.75	42	Achlorhydric swine gastric juice + Vegex	0.8
May 18.....			" " " " "	0.2
May 19.....			" " " " "	1.0
May 20.....			" " " " "	0.4
May 21.....			" " " " "	0.2
May 22.....	1.48	36	" " " " "	0.4
May 23.....			" " " " "	0.4
May 24.....	1.41	33	" " " " "	1.0
May 25.....			" " " " "	0.8
May 26.....	1.46	34	Normal swine gastric juice + Vegex	0.2
May 27.....			" " " " "	0.6
May 28.....			" " " " "	1.0
May 29.....	1.09	28	" " " " "	0.4
May 30.....			" " " " "	0.8
May 31.....	1.14	26	" " " " "	2.0
June 1.....			" " " " "	8.4
June 2.....	1.09	25	" " " " "	22.4
June 3.....			" " " " "	28.2
June 4.....			" " " " "	23.2
June 5.....	1.73	38	" " " " "	12.0
June 6.....			" " " " "	7.0
June 7.....	1.98	42	" " " " "	
June 8.....			" " " " "	
June 9.....	2.06	44	" " " " "	
June 10.....			" " " " "	

The extracts were given intravenously over a period of ten days, during which red blood cell and reticulocyte levels were followed closely. Following this period of therapy, liver extract made by the same method from the livers of normal pigs was substituted and the blood studies continued.

In Figure 11 and Table VI, are presented the

TABLE VI

The changes in blood levels of Patient 2, E. K., with pernicious anemia during the injection of extracts of the livers of anemic and of normal swine.

Date	R.B.C.	Hgb.	Therapy (intravenous)		Reticulocytes
1938	millions	per cent	Liver extract	Dose	per cent
September 8...	1.89	57	Anemic swine	10	0.6
September 9...			" "	10	0.6
September 10...			" "	10	0.4
September 11...			" "	10	0.2
September 12...	1.78	52	" "	10	0.2
September 13...			" "	10	0.6
September 14...			" "	10	0.2
September 15...	1.80	46	" "	10	0.4
September 16...			" "	10	0.4
September 17...			" "	10	0.6
September 18...			Normal swine	10	0.4
September 19...			" "	10	0.8
September 20...			" "	10	2.0
September 21...			" "	10	2.0
September 22...			" "	10	2.2
September 23...	2.18	50	" "	10	3.0
September 24...			" "	10	3.0
September 25...			" "	10	4.2
September 26...	2.12	58	" "	10	14.2
September 27...			" "	10	21.2
September 28...			" "	10	12.8
September 29...	2.57	63	Parke Davis	20	7.6
September 30...			" "	20	3.2
October 1.....			" "	20	2.0
October 2.....			" "	20	
October 3.....	2.97	75	" "	20	
October 4.....			" "	20	
October 5.....			" "	20	
October 6.....	3.69	74	" "	20	

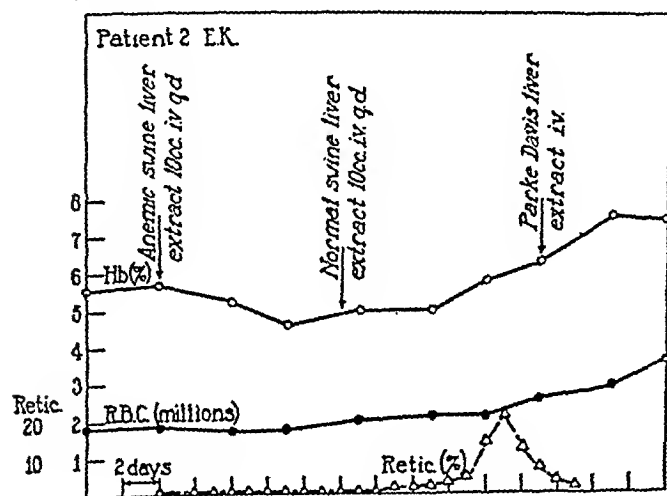


FIG. 11. A GRAPH OF THE CHANGES IN BLOOD LEVELS OF A PATIENT WITH PERNICIOUS ANEMIA DURING THE ADMINISTRATION OF EXTRACTS OF ANEMIC AND NORMAL SWINE LIVERS.

results of one such experiment (Patient 2). The patient, over a ten-day period, received intravenously an amount of extract derived from 500 grams of whole liver. At the beginning of the study the erythrocyte count was 1,890,000, the hemoglobin 57 per cent, and the reticulocyte count 0.6 per cent. During this entire first ten-day period the red blood cell count did not change essentially, nor was an increase of reticulocytes observed. On the eleventh day the patient was given, by the same route, a similar amount of liver extract made by the same procedure, but from the livers of normal swine. At this time the red blood cell count was 2,060,000, and the hemoglobin 50 per cent. On the sixth day the reticulocyte count rose to 3 per cent, on the seventh day to 4.2 per cent, on the eighth day to 14.2 per cent, and on the ninth day to 21.2 per cent. Following this, the red blood cell count rose on the eleventh day to 2,570,000, and the hemoglobin to 63 per cent. The patient received in all an amount of liver extract derived from 400 grams of normal swine liver.

In Figure 12 and Table VII, are presented the blood studies of Patient 3. The experiment was similar to the one just described except that the injections were given for thirteen days and an amount of liver extract derived from 650 grams of liver was administered. At the beginning of this first period the red blood cell count was 2,200,000, the hemoglobin 63 per cent, and the reticulocyte count 0.8 per cent. During the en-

TABLE VII

*The changes in blood levels of Patient 3, A. L., with pernicious anemia during the injection of extracts of the livers of anemic and normal swine.*

Date	R.B.C.	Hgb.	Therapy (Intravenous)		Reticulocytes
			Liver extract	Dose	
1933	millions	per cent		cc.	per cent
September 16..	2.20	63	Anemic swine	10	0.8
September 17..			" "	10	0.6
September 18..			" "	10	0.6
September 19..			" "	10	1.0
September 20..	2.09	65	" "	10	0.6
September 21..			" "	10	0.8
September 22..			" "	10	0.5
September 23..	2.17	65	" "	10	1.2
September 24..			" "	10	1.0
September 25..			" "	10	0.6
September 26..	2.21	60	" "	10	0.8
September 27..			" "	10	1.2
September 28..			" "	10	1.8
September 29..	1.63	51	Normal swine	10	1.6
September 30..			" "	10	1.4
October 1.....			" "	10	2.8
October 2.....			" "	10	5.2
October 3.....	2.18	62	" "	10	5.8
October 4.....			" "	10	9.2
October 5.....	2.48	64	" "	10	7.4

tire period of thirteen days there was no improvement in the blood values and no rise in the reticulocyte count. On the second day after the injection of normal pig liver extract, the reticulocytes rose to 2.8 per cent, on the fourth day to 5.8 per cent, and on the fifth day to 9.2 per cent. This was followed by an improvement of the blood values, the red count rising to 2,480,000 and hemoglobin to 64 per cent. An amount of liver extract derived from 300 grams of normal swine liver was given.

From the experiments described, it appears that the livers of the swine which developed the syndrome of achlorhydria, anemia and mouth lesions did not contain the anti-anemia substance which is present in the livers of normal swine.

#### DISCUSSION

From the experimental evidence presented, it appears that by feeding to swine a diet which causes canine black-tongue, a characteristic symptom-complex can be produced. Macrocytic anemia, lesions of the oral mucous membrane, gastric achlorhydria, diarrhea and motor weakness of the extremities were the principal manifestations. When the disease was present it was impossible to demonstrate hematopoietic activity of the gastric secretion or liver when tested on cases of pernicious anemia in man. The gastric secretion

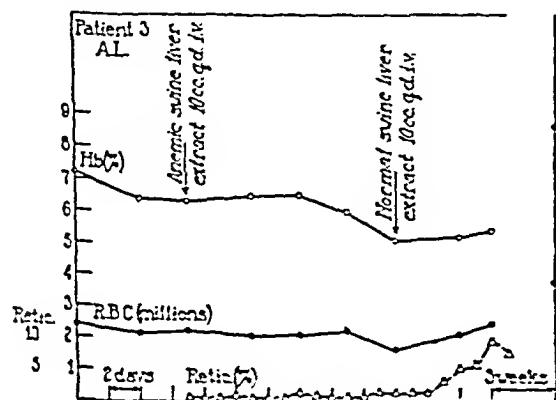


FIG. 12. GRAPH OF THE CHANGES IN BLOOD LEVELS OF PATIENT 3 WITH PERNICIOUS ANEMIA DURING THE INJECTION OF EXTRACTS OF THE LIVERS OF ANEMIC AND NORMAL SWINE.

and livers of normal swine did possess hematopoietic power when tested in exactly the same manner.

The disease syndrome of swine could be prevented and alleviated by the administration of certain substances which are prophylactic and curative of sprue, tropical macrocytic anemia, pellagra and pernicious anemia in man. The symptomatic, pathologic, physiologic and therapeutic similarity between the experimental disease of swine and a group of pathologic states of human beings was striking; so much so, that the inference is unavoidable that in man the pathologic changes are due, in part at least, to a common cause, and that the cause is basically a lack of intake or utilization of some as yet poorly defined dietary constituent. Certain objections to this broad conclusion exist, however, and several details require further discussion.

As previously stated, the anemia produced was usually sharply macrocytic in type, presenting morphological changes seen in the stained blood smear which were consistent with those shown by the blood of the macrocytic anemias of man. In several instances, however, microcytosis was the rule, but in such instances the hypochromia was never of the marked degree observed in experimental anemias resulting from a lack of the building stones of the hemoglobin molecule such as iron or copper. It more closely resembled the mild microcytosis frequently observed early in the course of tropical sprue and more rarely late in the course of that disease when a multiple deficiency is present (Castle and Rhoads (1)).

In the experiments, distinct therapeutic responses were obtained by the administration of liver extract, whereas none followed the use of iron alone. That better responses might have been induced had both substances been administered can only be the subject of speculation, since the observations were confined to the use of one or the other material alone. A summation response to the administration of liver extract and iron is occasionally seen in pernicious anemia and frequently in tropical sprue.

The distinct curative effect following the use of liver extract, either orally or parenterally administered, is in keeping with numerous observations on the effect of liver extract in the pre-

vention of symptoms when the same diet is fed to dogs (Goldberger and Sebrell (22), Rhoads and Miller (2)).

The changes occurring in the oral mucous membranes deserve special attention. An atrophic glossitis similar to that produced in dogs, and seen in sprue, pellagra and pernicious anemia, was observed in only rare instances. In no animal did it approach in intensity either the canine or the human disease. On the other hand, localized ulcerated lesions of the labial, buccal, lingual, and even laryngeal mucous membrane, were striking in their frequency and extent. For the purpose of discussion, it has been assumed that these lesions are similar in nature to the aphthous stomatitis with ulceration which is so marked a feature of tropical sprue. The histopathology of these changes will be described elsewhere; it suffices to state here that the process is essentially one of coagulation necrosis, apparently similar in nature to, though more localized than, the changes described by Lillie (23) in canine black-tongue. No studies of the aphthous oral lesions of tropical sprue are available for comparison.

As presented in the experimental protocols, one animal showed an acute gastritis at postmortem examination. In other animals there appeared to be a definite loss of thickness with atrophy of the gastric mucous membranes. Histological studies were not wholly satisfactory, since the unavoidable delay intervening between the time of the animal's death and the tissue fixation was sufficient to allow a certain amount of postmortem change to take place. Further investigation of this phase of the problem is proceeding. Clear morphological evidence of alteration of, or damage to, the gastric mucous membrane has certainly not been demonstrated consistently. Evidence of functional damage to the gastric secretory mechanism appeared in almost every experiment, however. Such evidence was at hand in the development of the inability of the stomach to secrete free HCl, and also to secrete a ferment required for hematopoiesis.

Achlorhydria, present even after the injection of histamine, was an early and persistent symptom. It disappeared after treatment in one instance. It is quite impossible, in the present state of knowledge, to speculate concerning the cause

of this manifestation. It can only be stated that to judge from the studies of the effect of feeding the same diet to other animals the achlorhydria was not due to a lack of any of the known fractions of the vitamin B complex.

The loss of the hematopoietic power of the stomachs of the experimental animal was demonstrated in three experiments, one involving a direct test, and two demonstrating the loss of the presumed stored product of the hematopoietic gastric function in the liver. These experiments require some explanation. Clinical study has shown that in pernicious anemia, and in some cases of sprue, the gastric secretion lacks a ferment which is required to act upon a dietary constituent present in yeast, meat and other substances, to form a substance active in hematopoiesis. In the absence of this gastric activity, the liver is no longer potent in promoting blood formation, Maisson and Ivy (7). Hence, test of the hematopoietic function of the liver is indirectly a test of gastric function, and may be more easily performed than the latter. This is due to the fact that potent liver extracts are heat resistant and so may be sterilized and injected into suitable test patients, a procedure not possible with extracts of gastric tissue. In all three of the tests done, a complete lack of hematopoietic function of the diseased animals was shown, a striking contrast to the results of tests of like material prepared from normal animals. The uniform nature of the results suggests definitely that, under the conditions observed, achlorhydria and loss of the anti-pernicious anemia activity of the stomach may follow a deficiency of a specific dietary constituent. That constituent is one which is contained in those substances which are therapeutically active in symptomatically similar conditions of man. Lastly, clinical observation offers incomplete but undeniable evidence that in man similar manifestations may follow deficient intake or absorption of the same substances.

From the reported studies of the modified black-tongue-producing diet, the cause of its pathogenicity is not clear. Since the power of promoting the growth of young rats kept under standard conditions is the only available means of determining the presence or absence of the various fractions of the vitamin B complex, we are forced to conclude

that the diet is adequate in its content of that vitamin. The objection may be advanced that the rat and the dog may differ radically in their requirement for vitamin B, and hence a diet which is wholly adequate for one animal might be a deficient diet for another. This objection would be valid were it not for the fact that neither in other laboratories nor in the control experiments reported here has black-tongue resulted from feeding diets complete except for the vitamin B complex. Zimmerman and Burack (14) and Bliss (24) have suggested that the diet lacks certain mineral constituents. Opposed to this view is the fact that no experimentally produced mineral deficiency has been reported as causing the symptoms of black-tongue. Furthermore, the symptoms may be prevented by supplementing the diet with a compound such as liver extract, which is exceedingly low in mineral constituents.

The assumption has been widespread that because the symptoms resulting from the feeding of a particular diet are prevented or cured by administering a material which is rich in a particular vitamin, it may be inferred that the symptoms are due to a lack of that vitamin. Since our knowledge of the mode of action of vitamins is seriously deficient, and since in the experiments under discussion an even relatively pure source of vitamin was not available, any conclusion as to the etiological rôle played by a particular vitamin deficiency must be open to question. In the studies reported here, the substances fed as sources of vitamin B<sub>2</sub> (G) contain an infinite variety of different compounds, among which that vitamin may be one of the least important. No claim for the etiologic or therapeutic activity of vitamin B<sub>2</sub> (G) is here advanced, and, moreover, it is held that no conclusive proof exists for the therapeutic activity of vitamin B<sub>2</sub> (G) in sprue, pellagra, pernicious anemia, tropical macrocytic anemia, or canine black-tongue. On the other hand, proof to the contrary is also lacking, so that the matter must be considered quite unsettled at present. The lack of complete understanding of the mode of production of the symptom-complex should not obscure the fact that the feeding of the black-tongue-producing diet gives rise to symptoms in animals which not only simulate pellagra in man, but also when studied under particular experi-

mental conditions simulate sprue and pernicious anemia.

#### SUMMARY AND CONCLUSIONS

1. By feeding a modified canine-black-tongue-producing diet to swine, a symptom-complex marked by oral mucous membrane lesions, achlorhydria and anemia may be caused.

2. The disease is associated with a loss of the anti-pernicious anemia activity of the gastric secretion and liver.

3. Remissions of the anemia and amelioration of symptoms may be induced by the oral or parenteral administration of liver extract.

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# APPLICABILITY OF REBREATHING METHOD FOR DETERMINING MIXED VENOUS $\text{CO}_2$ IN CASES OF CHRONIC PULMONARY DISEASE

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The carbon dioxide tension of oxygenated mixed venous blood has long been used, in normal individuals and frequently in subjects with various forms of disease, in the determination of cardiac output according to the Fick principle. The method of rebreathing mixtures of  $\text{CO}_2$  and oxygen, for establishing equilibrium between the  $\text{CO}_2$  tensions of the lungs and the incoming venous blood, and at the same time oxygenating this blood, was first described by Christiansen, Douglas and Haldane (1); later by Henderson and Prince (2) and others. In 1922 Douglas and Haldane (3) showed that the same arterio-venous differences in  $\text{CO}_2$  content were obtained in a given subject, whether the rebreathing procedure equilibrated  $\text{CO}_2$  tensions only and oxygenated the blood in the lungs, or whether this procedure equilibrated simultaneously both  $\text{CO}_2$  and oxygen tensions of incoming venous blood with the rebreathed air. Field, Bock, Gildea, and Lathrop (4) showed that arterial blood drawn during the course of rebreathing a mixture of 6 per cent  $\text{CO}_2$  and 94 per cent oxygen was fully oxygenated, and that the  $\text{CO}_2$  tensions in arterial blood and in the rebreathed air were the same. Most workers have been able to demonstrate an equilibrium of  $\text{CO}_2$  tensions by similar rebreathing technique; Hamilton, Moore and Kinsman (5), however, were unable to establish equilibrium or "plateau" levels of  $\text{CO}_2$  during rebreathing in experiments with a small number of subjects. Richards and Strauss (6) in 1930 reviewed the numerous assumptions made in various rebreathing methods for estimating the values of the gases of the mixed venous blood. They showed experimentally that the same equilibria or "plateau" levels of  $\text{CO}_2$  tension of oxygenated mixed venous blood could be regularly obtained in a normal subject after 15 to 20 seconds of re-

breathing, with the use of initial mixtures in the rebreathing bag which differed in  $\text{CO}_2$  tensions by 10 mm. or more. By constructing a nomogram of the subject's blood, and by plotting on this nomogram the  $\text{CO}_2$  tensions (as obtained by rebreathing) of (a) oxygenated and (b) true mixed venous blood, these authors found that the (a) and (b)  $\text{CO}_2$  tensions represented approximately the same  $\text{CO}_2$  content of the blood.

It is of interest to inquire how far the method of rebreathing can be applied to the determination of  $\text{CO}_2$  tensions in oxygenated mixed venous blood in cases with various forms of pulmonary disease. This inquiry forms the subject of the present paper. Specifically, answers have been sought, in each patient studied, to the following questions.

1. During the course of the rebreathing procedure can a constant or nearly constant ("plateau") level of  $\text{CO}_2$  tension be demonstrated, which persists over a five second interval, or longer (e.g., from 15 to 20 seconds after the start of rebreathing)?

2. If this is the case, can the same plateau level of  $\text{CO}_2$  tension be arrived at, in successive experiments, when the initial  $\text{CO}_2$  tensions in the rebreathing bag differ by several millimeters?

3. When such a transient equilibrium of  $\text{CO}_2$  tension in the rebreathed air is established, is there at the same time a close agreement between the tensions of  $\text{CO}_2$  and oxygen in the air sample taken at the end of expiration ("alveolar" air), and the  $\text{CO}_2$  and oxygen tension in the blood leaving the lungs (arterial blood)?

That the establishment of these three equilibria indicates that the plateau level of  $\text{CO}_2$  tension is the same as that of the oxygenated mixed venous blood, may be argued as follows:

The complete oxygenation of the arterial blood



mental conditions simulate sprue and pernicious anemia.

#### SUMMARY AND CONCLUSIONS

1. By feeding a modified canine-black-tongue-producing diet to swine, a symptom-complex marked by oral mucous membrane lesions, achlorhydria and anemia may be caused.

2. The disease is associated with a loss of the anti-pernicious anemia activity of the gastric secretion and liver.

3. Remissions of the anemia and amelioration of symptoms may be induced by the oral or parenteral administration of liver extract.

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of rebreathing, will be constantly receiving  $\text{CO}_2$  from the incoming venous blood, as well as from the rebreathed air; and the  $\text{CO}_2$  tension of oxygenated mixed venous blood is therefore more readily attained throughout the lungs, providing that ventilation is sufficient throughout to bring about complete arterial oxygenation. It is therefore possible that a method of rebreathing  $\text{CO}_2$  may be valid in cases where a method using inhalation of a foreign gas is not.

The question may well be raised, why equilibration for oxygenated mixed venous  $\text{CO}_2$  was attempted in the present investigation, rather than equilibration for mixed venous oxygen or for both true mixed venous  $\text{CO}_2$  and  $\text{O}_2$ , as in the technique of Burwell and Robinson (9). The chief reason for our choice of method was the results of Richards and Strauss (6), who demonstrated both on theoretical and experimental grounds, in normal subjects, the difficulty of securing adequate equilibrium of rebreathed gases, with respect to oxygen. Presumably this difficulty would be increased in subjects with disturbed pulmonary function. Grollman, Friedman, Clark and Harrison (10), in a recent paper, have brought out some of the practical difficulties of oxygen-equilibration methods. Friedman, Clark and Harrison (11) have subsequently described an adaptation of the Burwell-Robinson technique, modified by the use of longer rebreathing periods, repeated samplings during rebreathing, and arterial blood samples also drawn during rebreathing. It may be that this method will be found applicable to certain cases of pulmonary disease.

An important assumption should perhaps be mentioned, which is made both by methods employing the Fick-principle and by most methods using inhalation of a foreign gas (6, 12); namely, that any change in blood flow produced by the hyperventilation of rebreathing, does not measurably affect the arteriovenous differences of oxygen or  $\text{CO}_2$  within the time during which rebreathing takes place.

#### METHODS

The apparatus and technique were essentially the same as those employed by Richards and Strauss (6). A diagram is shown in Figure 1.

For the experiment, the subject was in the resting posture, lying supine, with one pillow. Mouthpiece and noseclip were applied and the subject respired

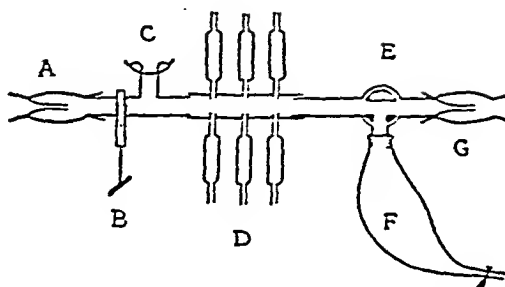


FIG. 1. DIAGRAM OF APPARATUS USED FOR OXYGENATED MIXED VENOUS  $\text{CO}_2$  TENSION DETERMINATIONS.

A, intake flutter valve. B, shut-off slide valve. C, mouthpiece. D, evacuated gas sampling tubes. E, three-way valve, connecting with rebreathing bag F. G, out-flow flutter valve. For cardiac output determination, the same apparatus was used, outside air being led in through valve A, and expired air from G collected in a Tissot spirometer.

through the apparatus for about ten minutes, so that a steady state might be reached. At a signal "blow," given at the end of a normal expiration, the subject made a complete expiration, while slide valve B was closed off. An alveolar sample was then taken into one of the evacuated tubes, and valve E then turned to connect the subject with the rebreathing bag. The subject then emptied and filled the bag completely, by successive respiration, a sample being taken into the evacuated sampling tubes every five seconds at the end of complete expiration. The rate of respiration varied with the subject; those with large vital capacities and slow resting respiratory rates made only four or five complete respirations in 20 seconds, those with small capacities and rapid rates rebreathed six or seven times in 20 seconds. The volume of air used in the bag for rebreathing also varied with each subject, from 1,500 cc. to 3,000 cc.; the volume being such that the subject could just empty the bag completely with a deep, rapid inspiration.

Several preliminary experiments were made during the first few days of the investigation of a given subject, with the double purpose of training him in the technique of rebreathing and of determining proper concentrations of  $\text{CO}_2$  to be used in the rebreathing bag. The remainder of the gas mixture was, of course, pure oxygen. Usually one experiment with a very high (over 60 mm.)  $\text{CO}_2$  concentration and one with a very low (less than 45 mm.) were performed to provide an approximate estimate of the level of  $\text{CO}_2$  tension reached by the subject during rebreathing. In succeeding experiments, intermediate concentrations of  $\text{CO}_2$  were used, and the upper and lower limits were thus determined within which the rebreathing procedure brought the  $\text{CO}_2$  tension to a constant level.

For the exact determination of oxygenated mixed venous  $\text{CO}_2$ , two or more experiments were performed on the same morning, and the  $\text{CO}_2$  tension of the mixture of  $\text{CO}_2$  and oxygen in the bag, as measured just before the

during rebreathing shows that all parts of the lungs which are perfused with pulmonary blood are at least partially ventilated. The close agreement between the  $\text{CO}_2$  tension of the alveolar air with that of the arterial blood during rebreathing indicates that there are no large regions of the lungs in which the  $\text{CO}_2$  of the regional, true alveolar air is appreciably different from the  $\text{CO}_2$  of the "alveolar" (expired air) sample as measured. If there were such regions the arterial blood sample taken during rebreathing would have a  $\text{CO}_2$  tension different from that of the "alveolar" sample as measured, except for an improbable or occasional situation in which regional variations in  $\text{CO}_2$  tension of alveolar air or pulmonary capillary blood happened to result in a mean arterial tension identical with that in a sample of the expired air. If mixing of rebreathed air throughout the lungs and the rebreathing bag is complete, and if the same  $\text{CO}_2$  tension after rebreathing is reached within the circulation time of the blood in successive experiments when the initial tension of  $\text{CO}_2$  rebreathed in each experiment is different, there is no reasonable explanation other than that the excess of  $\text{CO}_2$  has been carried away by the blood, or the needed deficit of  $\text{CO}_2$  supplied by the incoming blood, and that the state of equilibrium means an essential identity between  $\text{CO}_2$  tension in the alveolar air and in the oxygenated mixed venous blood. There may actually be certain small systematic differences, due to recirculation of small amounts of blood (such as that of the coronary circulation), or to continuous concentration of the rebreathed air due to absorption of oxygen; these factors have been discussed by Richards and Strauss (6) and need not be reviewed further. A false equilibrium or "plateau" level may also be obtained by the use of a very high concentration of  $\text{CO}_2$  in the rebreathed air, as shown both on theoretical and experimental grounds by the same workers; but there would be in this case no identity of equilibrium with respect to  $\text{CO}_2$  tensions, when different initial mixtures of  $\text{CO}_2$  were used.

The equality of the levels of equilibrium on successive rebreathings also indicates that the individual studied is in a steady state with respect to his mixed venous blood gases.

In abnormally functioning lungs, the rebreathing process may not provide complete mixing

between true alveolar air and that in the rebreathing bag, as for instance in a case with large volume of residual air, or one with a small or poorly distributed tidal air. Does the establishment of the three equilibria above mentioned indicate even in this case that the level of the plateau of  $\text{CO}_2$  tension is the same as the level of  $\text{CO}_2$  tension of the oxygenated mixed venous blood? With respect to the equilibrium which does exist between the  $\text{CO}_2$  tension of the sample of expired or "alveolar" air, and the  $\text{CO}_2$  tension of the sample of arterial blood drawn during rebreathing, the same argument that has just been given holds true: namely, that the  $\text{CO}_2$  tension of the sample of expired air represents at least the resultant or mean of the  $\text{CO}_2$  tensions in the pulmonary capillaries. If equilibrium with respect to  $\text{CO}_2$ , between incoming blood and alveolar air did not exist, there would be a general trend of change of  $\text{CO}_2$  tension in successive samples of expired air, toward the level in the blood, though with incomplete mixture such a trend might be at times irregular. Thus, so far as concerns the successive  $\text{CO}_2$  tensions of expired air, measured at 5 second intervals during rebreathing, it is quite possible that incomplete mixture might result in irregularities in the curve of change of these values, and that false "plateau" levels might appear in the curve. In this case, however, it is unlikely that such apparent equilibria would occur regularly over the same time interval; and still more unlikely that with different initial mixtures of  $\text{CO}_2$  in the rebreathing bag, the same equilibrium should be thus reached in successive experiments over the same time intervals.

From the argument just given it seems a reasonable conclusion that if the three equilibria above outlined are established consistently in a given case, then the  $\text{CO}_2$  tension so defined is close to that of the oxygenated mixed venous tension. The question of the magnitude of the error involved in the determination will be discussed after the data are presented.

It should be noted that in the process of equilibration by rebreathing for the determination of oxygenated mixed venous  $\text{CO}_2$  tension, there is an inherent advantage not shared by a technique employing inhalation of a foreign gas; in that poorly ventilated parts of the lungs, during the process

of rebreathing, will be constantly receiving  $\text{CO}_2$  from the incoming venous blood, as well as from the rebreathed air; and the  $\text{CO}_2$  tension of oxygenated mixed venous blood is therefore more readily attained throughout the lungs, providing that ventilation is sufficient throughout to bring about complete arterial oxygenation. It is therefore possible that a method of rebreathing  $\text{CO}_2$  may be valid in cases where a method using inhalation of a foreign gas is not.

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An important assumption should perhaps be mentioned, which is made both by methods employing the Fick-principle and by most methods using inhalation of a foreign gas (6, 12); namely, that any change in blood flow produced by the hyperventilation of rebreathing, does not measurably affect the arteriovenous differences of oxygen or  $\text{CO}_2$  within the time during which rebreathing takes place.

#### METHODS

The apparatus and technique were essentially the same as those employed by Richards and Strauss (6). A diagram is shown in Figure 1.

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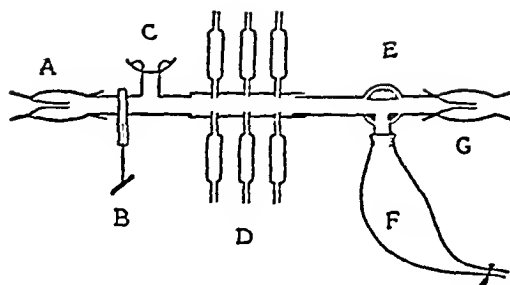


FIG. 1. DIAGRAM OF APPARATUS USED FOR OXYGENATED MIXED VENOUS  $\text{CO}_2$  TENSION DETERMINATIONS.

A, intake flutter valve. B, shut-off slide valve. C, mouthpiece. D, evacuated gas sampling tubes. E, three-way valve, connecting with rebreathing bag F. G, out-flow flutter valve. For cardiac output determination, the same apparatus was used, outside air being led in through valve A, and expired air from G collected in a Tissot spirometer.

through the apparatus for about ten minutes, so that a steady state might be reached. At a signal "blow," given at the end of a normal expiration, the subject made a complete expiration, while slide valve B was closed off. An alveolar sample was then taken into one of the evacuated tubes, and valve E then turned to connect the subject with the rebreathing bag. The subject then emptied and filled the bag completely, by successive respiration, a sample being taken into the evacuated sampling tubes every five seconds at the end of complete expiration. The rate of respiration varied with the subject; those with large vital capacities and slow resting respiratory rates made only four or five complete respirations in 20 seconds, those with small capacities and rapid rates rebreathed six or seven times in 20 seconds. The volume of air used in the bag for rebreathing also varied with each subject, from 1,500 cc. to 3,000 cc.; the volume being such that the subject could just empty the bag completely with a deep, rapid inspiration.

Several preliminary experiments were made during the first few days of the investigation of a given subject, with the double purpose of training him in the technique of rebreathing and of determining proper concentrations of  $\text{CO}_2$  to be used in the rebreathing bag. The remainder of the gas mixture was, of course, pure oxygen. Usually one experiment with a very high (over 60 mm.)  $\text{CO}_2$  concentration and one with a very low (less than 45 mm.) were performed to provide an approximate estimate of the level of  $\text{CO}_2$  tension reached by the subject during rebreathing. In succeeding experiments, intermediate concentrations of  $\text{CO}_2$  were used, and the upper and lower limits were thus determined within which the rebreathing procedure brought the  $\text{CO}_2$  tension to a constant level.

For the exact determination of oxygenated mixed venous  $\text{CO}_2$ , two or more experiments were performed on the same morning, and the  $\text{CO}_2$  tension of the mixture of  $\text{CO}_2$  and oxygen in the bag, as measured just before the

during rebreathing shows that all parts of the lungs which are perfused with pulmonary blood are at least partially ventilated. The close agreement between the  $\text{CO}_2$  tension of the alveolar air with that of the arterial blood during rebreathing indicates that there are no large regions of the lungs in which the  $\text{CO}_2$  of the regional, true alveolar air is appreciably different from the  $\text{CO}_2$  of the "alveolar" (expired air) sample as measured. If there were such regions the arterial blood sample taken during rebreathing would have a  $\text{CO}_2$  tension different from that of the "alveolar" sample as measured, except for an improbable or occasional situation in which regional variations in  $\text{CO}_2$  tension of alveolar air or pulmonary capillary blood happened to result in a mean arterial tension identical with that in a sample of the expired air. If mixing of rebreathed air throughout the lungs and the rebreathing bag is complete, and if the same  $\text{CO}_2$  tension after rebreathing is reached within the circulation time of the blood in successive experiments when the initial tension of  $\text{CO}_2$  rebreathed in each experiment is different, there is no reasonable explanation other than that the excess of  $\text{CO}_2$  has been carried away by the blood, or the needed deficit of  $\text{CO}_2$  supplied by the incoming blood, and that the state of equilibrium means an essential identity between  $\text{CO}_2$  tension in the alveolar air and in the oxygenated mixed venous blood. There may actually be certain small systematic differences, due to recirculation of small amounts of blood (such as that of the coronary circulation), or to continuous concentration of the rebreathed air due to absorption of oxygen; these factors have been discussed by Richards and Strauss (6) and need not be reviewed further. A false equilibrium or "plateau" level may also be obtained by the use of a very high concentration of  $\text{CO}_2$  in the rebreathed air, as shown both on theoretical and experimental grounds by the same workers; but there would be in this case no identity of equilibrium with respect to  $\text{CO}_2$  tensions, when different initial mixtures of  $\text{CO}_2$  were used.

The equality of the levels of equilibrium on successive rebreathings also indicates that the individual studied is in a steady state with respect to his mixed venous blood gases.

In abnormally functioning lungs, the rebreathing process may not provide complete mixing

between true alveolar air and that in the rebreathing bag, as for instance in a case with large volume of residual air, or one with a small or poorly distributed tidal air. Does the establishment of the three equilibria above mentioned indicate even in this case that the level of the plateau of  $\text{CO}_2$  tension is the same as the level of  $\text{CO}_2$  tension of the oxygenated mixed venous blood? With respect to the equilibrium which does exist between the  $\text{CO}_2$  tension of the sample of expired or "alveolar" air, and the  $\text{CO}_2$  tension of the sample of arterial blood drawn during rebreathing, the same argument that has just been given holds true: namely, that the  $\text{CO}_2$  tension of the sample of expired air represents at least the resultant or mean of the  $\text{CO}_2$  tensions in the pulmonary capillaries. If equilibrium with respect to  $\text{CO}_2$ , between incoming blood and alveolar air did not exist, there would be a general trend of change of  $\text{CO}_2$  tension in successive samples of expired air, toward the level in the blood, though with incomplete mixture such a trend might be at times irregular. Thus, so far as concerns the successive  $\text{CO}_2$  tensions of expired air, measured at 5 second intervals during rebreathing, it is quite possible that incomplete mixture might result in irregularities in the curve of change of these values, and that false "plateau" levels might appear in the curve. In this case, however, it is unlikely that such apparent equilibria would occur regularly over the same time interval; and still more unlikely that with different initial mixtures of  $\text{CO}_2$  in the rebreathing bag, the same equilibrium should be thus reached in successive experiments over the same time intervals.

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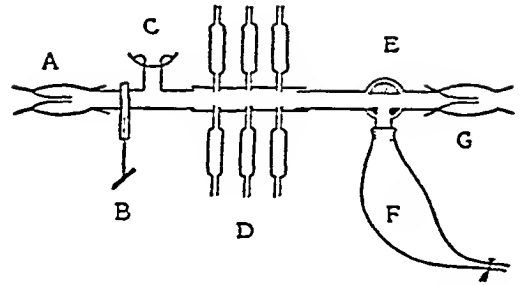


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scriptions of some of the cases are given in a subsequent paper.

E. S., an Italian-American youth of 17, with an early tuberculous lesion in the left upper lobe, was studied before and during the course of establishment of a left pneumothorax. He had, as shown in Table I, an excellent capacity for rebreathing to a constant level varying initial tensions of  $\text{CO}_2$  both before and after his pneumothorax. Furthermore, there was a close agreement (Table II) between rebreathed  $\text{CO}_2$  tensions in the expired air and the  $\text{CO}_2$  tension of the simultaneously drawn arterial blood; this experiment was done at a time when the patient had a complete left pneumothorax. Similar results were obtained with M. P., an Italian woman of 22, with a small healed lung abscess and a partial collapse, which was later allowed to re-expand; with the exception of a rather poor agreement between the  $\text{CO}_2$  in the lungs and the blood in the first experiment (Table II), although there was complete oxygenation of the arterial sample. In the second experiment a good agreement was obtained.

L. P. and I. A. were young men each of whom developed a spontaneous pneumothorax with positive pleural pressure (+4, +8 in L. P., +5, +13 in I. A.). Each one also had a partial re-expansion, then a second spontaneous collapse and a second re-expansion while under observation. L. P. showed (Table I) at first a satisfactory equilibration to constant  $\text{CO}_2$  tension in the rebreathing experiments, then a period (experiments of April 11th and 12th) following his second collapse when equilibration of  $\text{CO}_2$  was less satisfactory; followed in turn by a third period when positive pleural pressure was reduced and re-expansion progressing, during which satisfactory equilibration was again attained. No blood experiment was done with this patient. The arterial blood experiment on I. A., done when pneumothorax was still nearly complete and pleural pressure positive (+5, +13), showed a fairly good agreement of  $\text{CO}_2$  tensions in the lungs and the arterial blood, though oxygen saturation was not quite complete.

Patient J. H., with advanced bilateral tuberculosis, showed, nevertheless, surprisingly good capacity for bringing varying mixtures of  $\text{CO}_2$  and oxygen to an equilibrium during rebreathing. He had at this time a slight arterial oxygen unsatura-

tion. Following a partial pneumothorax, when arterial oxygen saturation was only 87 per cent, he was not able to equilibrate  $\text{CO}_2$  tensions by rebreathing quite as satisfactorily as before (Table I, April 20th). At this time an arterial blood experiment showed incomplete arterial oxygen saturation, and an arterial  $\text{CO}_2$  tension differing by 1.7 mm. from the corresponding  $\text{CO}_2$  tension of the alveolar air sample.

J. M., a patient with pulmonary fibrosis, markedly dyspneic but without arterial oxygen unsaturation, or  $\text{CO}_2$  "retention," had a good capacity for equilibrating a mixture of  $\text{CO}_2$  and oxygen by rebreathing; and equilibrium between lungs and blood during rebreathing could also be demonstrated. His respiratory state was, however, most unstable; this is suggested by the widely different levels of equilibrium, in respect to  $\text{CO}_2$  tensions, reached in successive rebreathing experiments (Table I). A large amount of other work on this patient being published elsewhere has given ample proof of this fact.

The last two cases, H. E. and L. C., had pulmonary emphysema. With the former it was usually possible in rebreathing experiments to establish an equilibrium in  $\text{CO}_2$  tension; though in some experiments the results were quite irregular. It was not possible, in two arterial blood experiments (Table II), to demonstrate the existence of equilibrium between tensions of the rebreathed expired air and the arterial blood. H. E. had arterial oxygen saturation, usually between 85 per cent and 90 per cent. With patient L. C., there was not demonstrable any equilibrium or "plateau" level of  $\text{CO}_2$  tension in the course of rebreathing; the curves of  $\text{CO}_2$  tensions of rebreathed expired air, plotted against time, as illustrated in Figure 2, and in the last experiment of Table I, are characteristic of the behavior of this emphysematous subject.

In brief, the experimental material shows: that in two cases of simple artificial pneumothorax, with pulmonary function otherwise good, and in one case of spontaneous pneumothorax with positive pleural pressure, it was possible to determine  $\text{CO}_2$  tensions of oxygenated mixed venous blood by rebreathing methods; that there was some doubt of the applicability of the method in one phase of a second case of spontaneous pneumothorax with positive pleural pressure; that the

E.S. Left Apical TB.

L.C. Emphysema

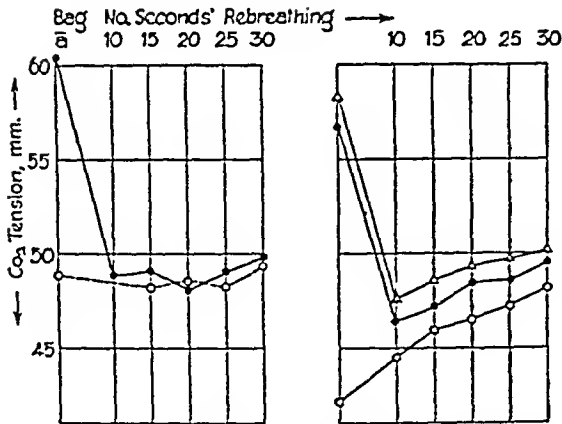


FIG. 2. CO<sub>2</sub> TENSIONS OF EXPIRED AIR DURING REBREATHING, SHOWING: EQUILIBRIUM REACHED BY E. S., ABSENCE OF EQUILIBRIUM LEVEL BY L. C.

First column (Bag *a*) gives initial CO<sub>2</sub> tension in rebreathing bag, before rebreathing was begun.

method was shown to be unreliable in a case of advanced bilateral tuberculosis with pneumothorax, in a case of pulmonary fibrosis with marked dyspnea, and in two cases of pulmonary emphysema.

It is probable that the CO<sub>2</sub> tensions obtained are not the absolute values for the CO<sub>2</sub> of the resting oxygenated mixed venous blood; but that there are small systematic errors due to recirculation of small amounts of blood within 20 seconds, to the hyperventilation of the rebreathing procedure, and so forth. These factors have been discussed previously by various investigators. The method thus gives a relative rather than absolute measure of this function.

An estimate can be made of the limits of experimental error of the method. For our own purposes, in cases of abnormal pulmonary function (see subsequent paper (13)), these values of CO<sub>2</sub> tension are useful only in determining the actual CO<sub>2</sub> content of the mixed venous blood. It may therefore be well to estimate the experimental error of measurement of this latter function. A group of two properly conducted rebreathing experiments with a trained subject can be expected to give three of the four values of CO<sub>2</sub> tension at the 15-second and 20-second intervals agreeing within about 0.7 mm. or 0.8 mm. of CO<sub>2</sub> (see Table I). This difference represents

about 0.3 volumes per cent of CO<sub>2</sub> content. In addition there is the experimental error of method in the level of the CO<sub>2</sub> dissociation curve on which these tensions are plotted; this is chiefly that due to the method of blood gas analysis, or plus or minus 0.1 volume per cent. These two sources thus constitute a considerable experimental error, of about 10 per cent in an ordinary determination of cardiac output, since the arterial arteriovenous differences of CO<sub>2</sub> with which one is usually dealing are only about 4 volumes per cent.

A final brief comparison may be suggested between the above method as applied to determination of cardiac output and the methods involving inhalation of a foreign gas. The errors of method are considerably less in the latter in cases with normally functioning lungs. On the other hand, it is probable that mixed venous CO<sub>2</sub> can be determined by rebreathing in certain cases of abnormal pulmonary function in which ventilation is not adequate for the determination of cardiac output by foreign gas methods. A further advantage of the technique which we have described is that it can determine the applicability or non-applicability of the method in a given case. This advantage is also shared, however, by the Grollman technique (10), through the measurement of acetylene in the blood.

#### SUMMARY

1. The applicability of the method of rebreathing for determining CO<sub>2</sub> tensions of the oxygenated mixed venous blood in certain cases of pulmonary disease has been investigated.

2. The method required the demonstration of the same "plateau" levels of CO<sub>2</sub> tension in successive rebreathing experiments, when the initial CO<sub>2</sub> tensions of the mixtures in the rebreathing bag in the separate experiments varied by several millimeters; and the demonstration of equilibrium between CO<sub>2</sub> tension in the samples of expired air (during rebreathing) and the CO<sub>2</sub> tension in the simultaneously drawn arterial blood.

3. It has been found that the method gave satisfactory results in two cases of unilateral artificial pneumothorax; gave doubtful results in two cases of spontaneous pneumothorax with positive pleural pressures; and was unreliable in a case of advanced bilateral tuberculosis, a case of pulmo-



nary fibrosis with dyspnea, and two cases of advanced pulmonary emphysema.

4. By the technique described it can be determined in a given case whether the method of re-breathing is applicable or not.

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## CARDIAC OUTPUT IN RELATION TO UNILATERAL PNEUMOTHORAX IN MAN

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The effects upon cardiac output of the establishment of a closed unilateral pneumothorax have been investigated from time to time in animals for many years past; but in man only very recently. The animal experimentation has had varying results, depending upon the type of experiment performed and the species of animal used (1, 5, 8). Most of them have been acute experiments, in which there has thus been no close similarity with the gradually induced and chronically maintained therapeutic pneumothorax. After a review of the literature, Weiss (1) concluded that in animals (such as rabbits) with imperforate mediastinum, the usual effect of unilateral pneumothorax is a decrease in total cardiac output, though he considered this to be due to secondary pulmonary and circulatory effects rather than to the pneumothorax itself. Perhaps the nearest morphological analogy have been the recent experiments of Hilton (2) on goats, a species whose mediastinum is apparently similar to that of man. Using animals of 20 to 25 kgm. weight, he found that a small pneumothorax (200 cc.) caused a small increase in cardiac output, associated with decrease in arterial oxygen saturation. A large pneumothorax (500 cc.) caused a fall in cardiac output, with further fall in oxygen saturation.

The first report that we have found of cardiac output measurements in man following the establishment of unilateral pneumothorax is that of Berconsky in 1931 (3). His method was essentially that of Field, Bock, Gildea and Lathrop (4), using  $\text{CO}_2$  tensions of the alveolar air as equivalent to  $\text{CO}_2$  tension of the arterial blood, and using the rebreathing technique to establish mixed venous  $\text{CO}_2$  tensions. He made three measurements on one case and one on a second; both were young women with nearly complete collapse of one lung. The three values for cardiac output in the first case averaged about 3.2 liters per minute, or less than half the average value obtained by this

method with normal subjects. In the one determination on the second case, the cardiac output was 7.4 liters per minute, or near the upper limit of his values for normal women. The method in this study provided no proof that alveolar air was equivalent to arterial blood in respect to  $\text{CO}_2$  tension, or that a definite equilibrium with respect to  $\text{CO}_2$  tension had been reached by rebreathing, in the technique for determining  $\text{CO}_2$  tensions of oxygenated mixed venous blood.

Richards, Riley and Hiscock (5) published in 1932 the results of determinations of cardiac output, also using a method involving the Fick principle, on three young men, before and at intervals during the establishment of unilateral pneumothorax. In one of these cases standard basal conditions were not maintained, and alveolar air samples only were used to determine values of arterial  $\text{CO}_2$  tension. The experiments on the other two cases were more carefully controlled. Standard basal conditions were observed. Arterial  $\text{CO}_2$  content was measured directly as well as indirectly through the use of  $\text{CO}_2$  tensions of alveolar air; in one case in every determination except one of the experiments in the control period, in the other case once during the control period and twice during the period when pneumothorax was being induced. In the determination of  $\text{CO}_2$  tensions of the oxygenated mixed venous blood, an equilibrium was established in successive rebreathing procedures, according to a technique previously described by Richards and Strauss (6) (also reviewed in the preceding paper by the present authors (7)). In one of the two patients, who had one lung approximately 50 per cent collapsed in one week, there occurred a diminution in cardiac output from an average of 5.0 liters per minute to an average of 3.3 liters per minute. The second case, one of whose lungs was about 70 per cent collapsed in a month, had a cardiac output at the end of this period of 4.0 liters per minute, as

compared with an average figure of 4.8 liters per minute in the control period.

During the past year Nylin (8) has published the results of cardiac output determinations on two carefully studied cases, before and during the course of establishment of unilateral pneumothorax. For the cardiac output determinations the acetylene method of Grollman was used. The first case was a man of 32, with a right lower lobe lesion. Practically complete collapse of the lung was obtained, with intrapleural pressure about neutral. Cardiac output varied between 4.6 and 3.7 liters per minute, but there was no consistent change after collapse of the lung was established. The second case was a woman with extensive tuberculosis of the left lung. Collapse was obtained in this case also, though high positive pleural pressures (+7, +9 and +12, +14) were maintained. In this case also there was no consistent change in cardiac output following pneumothorax, the values varying between 3.6 and 3.0 liters per minute.

The discrepancy in the results of the two papers just referred to calls for both examination of the technique employed and further study of the same subject. The method of Richards, Riley and Hiscock (5), even with the greatest care observed, involves a considerable experimental error, as further studies to be reported presently will show. An average decrease in cardiac output of 34 per cent in the first of the two cases and 17 per cent in the second would seem, however, to be outside these limits of error. When pneumothorax is established, as we will also show presently,  $\text{CO}_2$  tension of the alveolar air cannot be relied upon to represent the  $\text{CO}_2$  tension of the arterial blood; in the second case of Richards, et al., therefore, more numerous arterial blood studies should have been done. Since both of the cases had apparently normal pulmonary function, except for pneumothorax, it seems likely that the method of obtaining mixed venous tensions was adequate, if one may judge from the results of the previous paper by the present authors (7). However, as no studies were made of the  $\text{CO}_2$  and oxygen tensions of arterial blood drawn during the rebreathing procedure, this point remains unproved.

Nylin's use of the acetylene method raises the question of adequate mixture of this foreign gas through the lung fields in cases of pneumo-

thorax. No proof is presented that this took place, and it is of course an essential feature for proper application of the method (16). The recent studies of Björkman (9) show that collapsed lungs vary considerably in the amount by which they are aerated by respiratory movements.

In the present investigation we have made determinations of cardiac output on six cases with unilateral pneumothorax. Short case histories are given at the end of the paper. There were three cases of tuberculosis followed before and during pneumothorax treatment; one patient with healing lung abscess who was studied while she had a partial pneumothorax and again after complete re-expansion; and two patients with spontaneous pneumothorax, with at first strongly positive pleural pressures, on whom measurements were made at various stages of collapse. All the patients were afebrile throughout the periods of study.

#### METHOD

The technique was similar to that employed by Richards, Riley and Hiscock (5) but with more extended study of each individual case, and particular efforts to establish the validity of the arterial and venous  $\text{CO}_2$  values, upon which this cardiac output measurement is based.

Standard basal conditions were observed, the patient moving only from the ward to the laboratory in the morning, then resting for half an hour or longer on the bed. The patient lay in the supine position, with one pillow. Mouthpiece and noseclip were applied and he breathed through the apparatus for ten minutes in order that a steady state might be reached. A diagram of the apparatus used is shown in Figure 1 of our preceding paper (7). A six-minute sample of expired air was first collected in the spirometer. Then at the end of a normal expiration, at a signal "blow," a complete expiration was made by the subject, while slide valve *B* was shut off. At the end of complete expiration a sample of alveolar air was taken into one of the evacuated sampling tubes, and the slide valve then reopened. After a rest period of from three to five minutes the alveolar sampling was repeated; this time, immediately after the taking of the alveolar sample, valve *E* was turned to connect with the rebreathing bag. The subject then emptied and filled the bag with successive deep respirations, at the rate usually of one every 5 seconds, sometimes slightly more rapidly. In the early experiments a sample, at the end of a complete expiration, was taken into an evacuated tube after 15 seconds' rebreathing, then another respiration made, and valve *E* turned, closing off the rebreathing bag; a sample (20 seconds) was then taken from the bag. In the later experiments more evacuated sampling tubes were attached, and samples at the end of expiration were taken 15, 20, 25 and 30 seconds after the beginning of

rebreathing. The subject then had a rest period of 15 to 30 minutes after which the whole procedure just described was repeated.

Arterial blood samples (20 cc.) were drawn, while the subject respired through the apparatus, after infiltration with novocaine of the region of the brachial artery. With some subjects this was done before the rebreathing procedures, with others after, with others between the two periods. There appeared to be no difference, in this respect, in results obtained. There was no evidence that the puncture disturbed the steady state of the individual.

Gas analyses were done with the Haldane apparatus.

Special precautions were taken in handling the blood. It was drawn into an oiled syringe, transferred, under paraffin oil, to chilled bottles containing dried potassium oxalate and sodium fluoride, as described in our preceding paper. The blood was kept on ice until the analyses were done. Duplicate analyses of  $\text{CO}_2$  and  $\text{O}_2$  content were made, and, according to the "first method" of Austin, Van Slyke, et al. (11), two points were determined on the oxygenated whole blood  $\text{CO}_2$  curve. The tensions of the points were about 35 mm. and 55 mm. The curve was drawn on logarithmic paper, by the use of the linear relationship of Peters (18).

On this  $\text{CO}_2$  diagram, arterial, alveolar, and mixed venous points were then plotted, arteriovenous differences obtained, and cardiac output calculated in the usual manner by dividing arteriovenous  $\text{CO}_2$  difference into  $\text{CO}_2$  output per minute, and dividing this quotient by 10, to give cardiac output in liters. It was thought that arterial blood represented mixed venous blood in respect to water and electrolyte contents, more closely than would any peripheral venous sample.

In addition to the measurements incident to the determination of cardiac output, rebreathing experiments to determine mixed venous  $\text{CO}_2$  tensions were carried out from time to time during each patient's course, to insure so far as possible the applicability of the method. These have been described in our preceding paper (7). It will be noted that four of the subjects of the present investigation also had particular studies made of their mixed venous equilibria.

#### CRITICISM OF METHOD

Against cardiac output methods of the type described above, two general criticisms are usually made, and with justification. The first is that the various technical manipulations with blood and alveolar gases are so many, and the resulting arteriovenous difference of  $\text{CO}_2$  content so small that even with the most scrupulous technique the error of method will be large. We have discussed this in the preceding paper, in connection with determination of venous  $\text{CO}_2$  values, and concluded that this error will be at least 10 per cent, when

one includes both the level of the  $\text{CO}_2$  dissociation curve, and the mixed venous tensions.

The second criticism concerns the additional error that occurs in the determination of arterial  $\text{CO}_2$  content. Even when an arterial puncture is painless, there may be, as Jansen, Knipping and Stromberger (12) and others have shown, irregularities of pulse or respiration that may disturb the subject's steady state and so change the arterial  $\text{CO}_2$  level from its resting value. Work by numerous investigators has, however, shown that the  $\text{CO}_2$  tension of alveolar air and of arterial blood obtained during the same resting period, with trained subjects, are in agreement (13). This applies to average results; it is quite true that in individual experiments there may be differences of a millimeter or more. It is also true that with certain individuals a technique such as the Haldane-Priestley will give  $\text{CO}_2$  tensions of alveolar air that consistently differ by a millimeter or more from those of the arterial blood (14).

In cases of abnormal pulmonary function it is of course not justifiable to assume that  $\text{CO}_2$  tensions of alveolar air and arterial blood will correspond; in our tables we have included figures for cardiac output based upon arterial blood values only, except in control periods when no pneumothorax existed.

It should be noted also that since we do not know whether or not the acid-base relations of extravascular blood (oxalated or heparinized) exactly correspond with those of intravascular blood, arteriovenous differences as calculated have probably more of a relative than absolute significance.

From the above it is probably fair to conclude that in the use of this method repeated measurements in a given state are necessary, that to be significant one group of measurements should differ from another by well over 10 per cent, perhaps as much as 20 per cent.

It is far from an ideal technique, as it has a large error and is laborious and difficult. Its advantage in these cases of pulmonary abnormality is that by it one can obtain an indication in any given case whether the method is applicable or not.

Our interest in the present paper lies in the changes in cardiac output occurring in a given subject rather than in the absolute values of this function. Which of the various methods for cardiac

TABLE I

*Cardiac output and other circulatory and respiratory functions in relation to unilateral pneumothorax.*

Patient, sex, age and height	Date	Wt.  kgm.	Pul- monary ven- tila- tion  liters per min- ute	CO <sub>2</sub> out- put  cc. per min- ute	O <sub>2</sub> in- take  cc. per min- ute	Arterial blood			Al- veo- lar CO <sub>2</sub>  mm.	Oxygen- ated ven- ous CO <sub>2</sub>  mm.		A-V CO <sub>2</sub> difference		Cardiac output		Vital ca- pac- ity  liters	Ven- ous pres- sure  mm.	Pleural pressure  cm.	Lung col- lapse  per cent
						CO <sub>2</sub>	O <sub>2</sub>	O <sub>2</sub> sat- ura- tion per cent		vol- umes per cent	vol- umes per cent	arter- ial	alve- olar	arter- ial	alve- olar				
E. S. male 17 height 183 cm.	June 10		7.73	256	323	49.6	20.2	97	39.3	49.1	53.4	3.8	4.1	6.7	6.2	—	—	—	0
	June 15		7.28	243	301	—	—	—	39.1	49.9	53.7	—	4.5	—	5.4	—	—	—	0
	June 29	93	7.20	243	315	51.7	20.1	95	39.9	48.7	54.5	2.8	3.7	8.7	6.6	5.2	60	—	0
	July 12		7.99	219	277	47.2	20.1	96	35.4	46.9	52.0	4.8	5.5	4.6	—	—	90	—	0
	Aug. 5	86	8.57	224	318	49.4	16.6	93	34.1	48.0	54.4	5.0	6.4	4.5	—	—	—	—	20
	Sept. 21	86	7.20	217	296	51.9	18.8	98	36.4	47.4	57.2	5.3	4.9	4.1	—	—	95	+3, -8 +5, -1	70
M. P. female 22 height 142 cm.	Jan. 27		5.68	163	203	42.4	17.9	91	32.7	42.3	47.0	4.6	4.2	3.5	—	—	—	—	85
	Jan. 31		5.37	144	183	42.4	18.9	98	33.7	42.4	47.1	4.7	4.0	3.1	—	—	80	—	40
	Feb. 25		5.19	156	204	46.0	18.8	97	36.8	43.4	49.6	3.6	3.3	4.3	4.7	—	—	—	60
	Mar. 9	50	4.98	155	199	—	—	—	35.4	42.5	49.1	—	3.5	—	4.4	—	—	—	0
D. J. male 20 height 178 cm.	Apr. 18	67.3	6.49	214	238	50.8	18.5	96	37.9	49.7	55.2	4.4	4.5	4.9	4.8	4.1	—	—	0
	Apr. 22		6.29	195	223	50.2	18.8	95	39.1	49.7	54.0	3.8	4.3	5.1	4.5	—	60	—	0
	May 24		6.61	206	262	48.6	18.3	92	34.8	48.7	52.6	4.0	6.1	5.1	—	3.1	30	+1, -6	30
	June 3		7.00	195	247	43.9	18.3	94	32.3	45.6	48.7	4.8	6.4	4.1	—	2.9	125	+3, -2	40
F. P. male 26 height 180 cm.	Mar. 25	59.6	6.17	166	199	—	—	—	42.0	51.4	57.6	—	3.7	—	4.5	4.5	25	—	0
	Mar. 30		6.80	213	255	—	—	—	41.1	53.6	58.5	—	4.9	—	4.4	—	—	—	0
	Apr. 5	60.6	6.79	201	235	54.2	17.6	94	42.4	53.4	57.5	3.3	4.4	6.1	4.6	—	—	—	0
	Apr. 11		6.71	206	241	54.2	17.7	—	41.7	51.9	57.9	3.7	4.1	5.6	5.0	4.5	—	—	0
	Apr. 27	61.9	7.04	197	236	51.1	16.8	93	36.5	48.1	55.6	4.5	4.9	4.4	—	3.0	26	0, -4	40
	May 11	63.2	6.84	196	236	50.0	17.0	90	36.3	48.3	54.7	4.7	5.0	4.2	—	2.4	42	-1, -4	50
	June 7		6.56	180	230	49.7	18.3	93	36.6	49.1	53.3	3.6	5.9	5.0	—	2.8	70	+5, +1	—
	June 22	62.3	6.72	195	250	50.4	18.4	95	35.6	50.4	50.9	4.5	6.6	4.3	—	—	50	+3, +1	85
	July 30	59.6	6.90	177	230	48.2	18.7	93	34.0	48.1	52.5	4.3	6.3	4.1	—	2.2	50	+3, -2	95
	Oct. 11		7.55	199	263	49.5	17.3	94	35.5	49.3	54.0	4.5	5.8	4.4	—	—	—	—	90
I. A. male 24 height 180 cm.	Jan. 28	61.0	6.53	175	208	48.2	22.0	94	35.3	48.5	52.6	4.4	6.6	4.0	—	—	80	—	85
	Feb. 1		5.98	182	227	49.9	21.8	93	39.2	51.3	53.2	3.3	5.2	5.5	—	1.5	—	+8, +10	70
	Feb. 16		5.47	166	190	49.7	22.8	93	39.2	51.1	52.8	3.1	5.8	5.5	—	85	—	+4, +10	85
	Mar. 3		4.85	171	208	50.1	22.9	96	39.8	50.2	53.0	2.9	5.1	5.9	—	1.8	67	+5, -1	60
	Mar. 14	62.0	5.00	171	216	51.1	22.9	95	40.3	50.0	54.3	3.2	4.7	5.3	—	2.0	—	—	35
L. P. male 29 height 188 cm.	Mar. 6	61.0	6.00	215	259	46.9	20.5	95	38.5	48.2	51.6	4.7	3.9	4.6	—	2.1	105	+8, +1	80
	Mar. 10	62.9	5.41	207	244	48.1	20.9	95	40.4	47.1	52.3	4.2	2.7	4.9	—	2.4	45	+4, -2	65
	Mar. 28	64.1	6.32	212	260	47.7	20.9	93	38.9	45.7	51.7	4.0	2.9	5.3	—	1.7	78	+10, +3	85
	Apr. 14		5.90	205	292	47.0	20.0	93	38.9	46.8	53.3	6.3	3.2	3.3	—	2.1	60	+8, +1	75
	Apr. 25	65.6	5.54	210	256	48.5	21.4	98	38.8	46.4	52.0	3.5	3.6	6.0	—	2.6	67	+6, -2	65
	May 18	70.0	5.79	222	239	49.7	21.2	99	36.1	46.6	53.6	3.9	4.7	5.7	—	3.4	60	—	30

output determination gives values nearest the actual true ones is difficult to say. Earlier Fick-principle methods gave values in normal subjects that were almost certainly too high, due to inadequate technique for determining CO<sub>2</sub> or oxygen of the mixed venous blood (6). Later Fick-principle methods have given lower figures, in the region of 2.0 to 3.0 liters per minute per square meter of body surface (14). Starr's (15) recently published figures with a revised ethyl iodide inhalation technique are similar to these (2.40 ± .55 liters). The nitrous oxide method and the acetylene method have slightly lower normal values (2.2 ± .2 liters for the latter). The comparisons of Bauman and Grollman (16) between figures obtained by use of the acetylene technique and those obtained by direct puncture

of the right heart are valuable evidence, but were all apparently carried out on patients with some pathological condition. In four patients with presumably normal cardiac output this value varied between 2.2 and 3.3 liters per minute per square meter of body surface. Certain recent experiments with the acetylene technique suggest that this may give values that are too low: when for example, in a case of anemia Bandow, Birkner and Bohnenkamp (17) find arteriovenous oxygen differences that are 125 per cent of the subject's total oxygen capacity, one must necessarily question the reliability of the method in this instance. The most likely cause would seem to be recirculation of blood within the time of the experiment; Grollman himself (16) has carefully considered this possibility.

## RESULTS

The values for cardiac output in relation to pneumothorax and to several other circulatory and pulmonary functions determined at the same time for the six cases studied are given in Table I. In Figure 1 (A, B and C) the values for cardiac output of each case, in liters per minute per square meter of body surface, are plotted against the degree of collapse of the lung.

It will be seen that in the two cases most care-

fully studied and most adequately controlled (cf. preceding paper), Table I and Figure 1 (A), there was an unmistakable decrease in cardiac output while the subject had pneumothorax, partial or complete. One case was studied first during pneumothorax and later after reexpansion, the other patient was first studied before establishing pneumothorax. There was a good deal of variation in values during the control period in the latter patient; one of the measurements based on

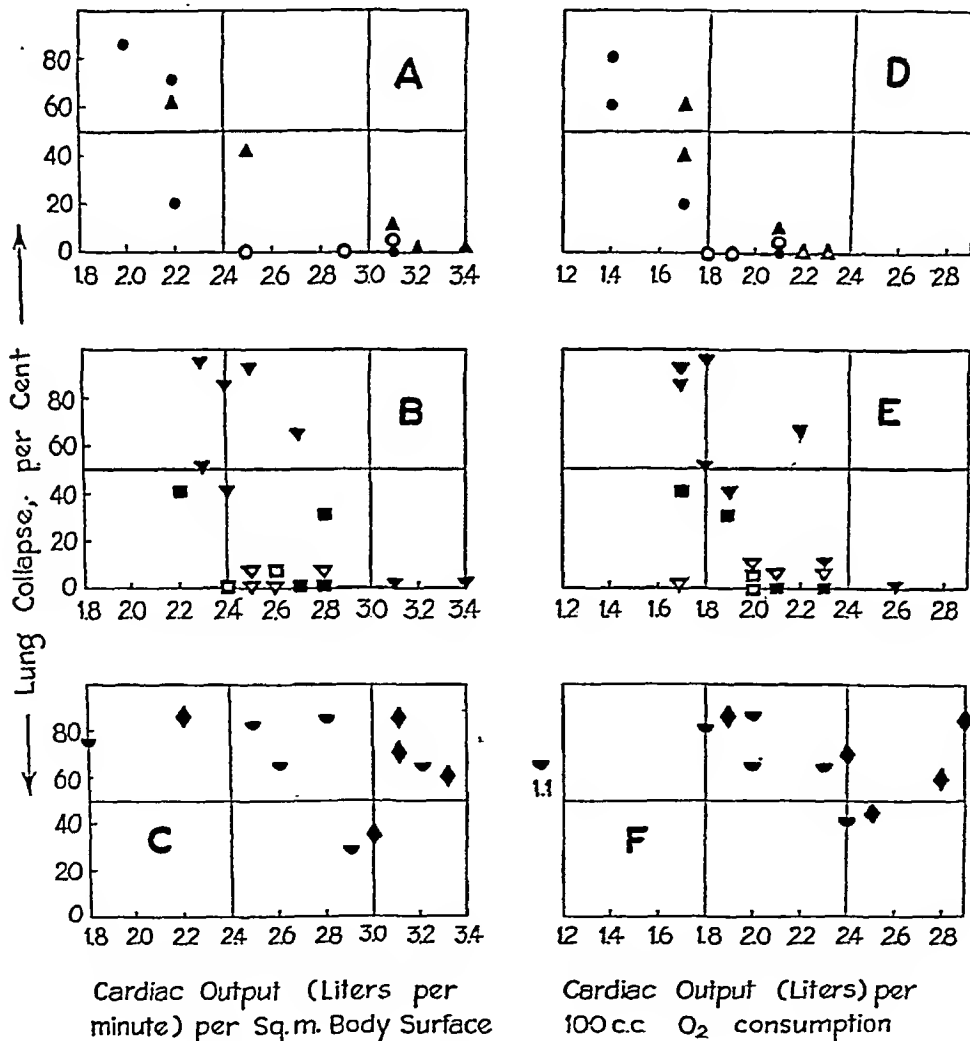


FIG. 1. CARDIAC OUTPUT RELATIONS IN PNEUMOTHORAX.

A and D: Subjects E. S. (●), M. P. (▲)

B and E: Subjects F. P. (▼), D. J. (■)

C and F: Subjects I. A. (♦), L. P. (♥)

Black symbols represent data calculated from arterial blood CO<sub>2</sub> contents, open symbols data based on alveolar CO<sub>2</sub> tensions.

arterial blood  $\text{CO}_2$  content was improbably high, and has been omitted from Figure 1.

In the other two cases of therapeutic pneumothorax, F. P. and D. J., studies of the equilibrium between rebreathed air and simultaneously drawn arterial blood were not made (see previous paper (7)), but as the other criteria were satisfactory, and pulmonary function except for the pneumothorax was good, it was thought that the technique employed was applicable. The results are shown in Figure 1 (B). The average cardiac output in F. P. per unit of body surface after pneumothorax was 14 per cent less than the average in the control period; but the scattering and overlapping of the values were considerable, and one cannot hold this difference to be significant. With D. J. no consistent change was found. There appeared to be a decrease in the last experiment with one lung collapsed at 40 per cent; unfortunately this patient left the hospital before further data were obtained.

Starr et al. (15), have shown a better correlation between cardiac output and oxygen consumption than between cardiac output and body surface. We have plotted this relationship in Figure 1 (D, E and F). Expressed in this way, the relative decrease in cardiac output during pneumothorax is brought out more clearly; even with patients F. P. and D. J. there is little overlapping of values determined during the pneumothorax as compared with the control period.

The two cases of spontaneous pneumothorax, as shown in Figure 1 (C and F) showed a wide variation of cardiac output, and no relation to the extent of pneumothorax. As discussed in our previous paper (7), further investigation of the mixed venous equilibrium in these two cases cast some doubt on the applicability of the method.

#### DISCUSSION

In the control state without pneumothorax the cardiac outputs of the cases E. S. and M. P. were somewhat higher than the average which we find by this method in normal individuals; in Figure 2 (A), for example, the average control value was 3.0 liters, that in Figure 2 (B) was 2.8 liters, which is about that usually obtained by this method in this age group. For subject E. S., the high control value is probably accounted for by

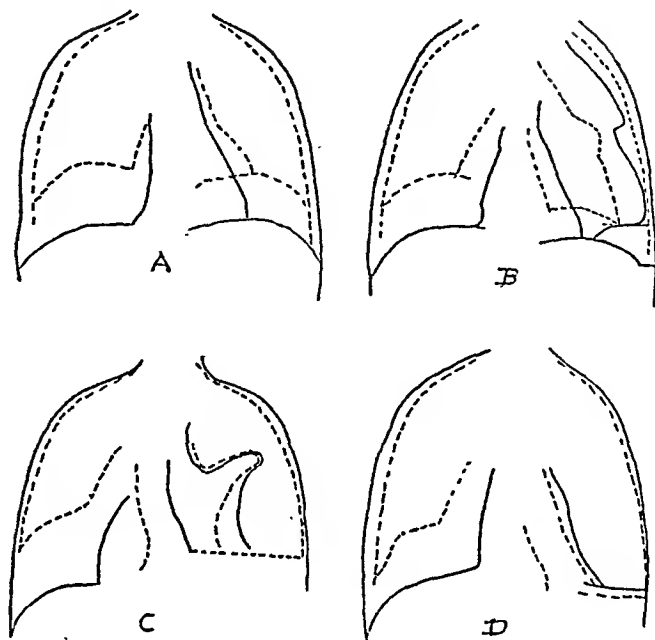


FIG. 2. X-RAY OUTLINES OF SUBJECT E. S. DURING DEEP INSPIRATION (CONTINUOUS LINES) AND DEEP EXPIRATION (DOTTED LINES), SHOWING PROGRESSIVE ESTABLISHMENT OF PNEUMOTHORAX.

A, June 29th; B, July 13th; C, August 4th; D, September 20th, 1932.

his age; Starr and his collaborators (15) have recently reported high values of cardiac output in the age group between 15 and 20 years. When expressed in terms of oxygen consumption per minute, it is found that all four of the cases of artificial pneumothorax had the same levels of cardiac output in the control periods (Figure 1, D and E). Even though the values in Group A in the control periods were slightly high, the cardiac output in the presence of pneumothorax was lower than the normal limits by this method for this age group.

It seems unlikely that initial unfamiliarity on the part of the subjects with the technique was a significant factor in the higher values for cardiac output in the control periods. The subjects had been trained in the technique before any of the reported measurements were made. Furthermore, subject M. P. was, as noted, studied first in the state of pneumothorax, and later in the re-expanded state.

The mechanism of this particular circulatory adaptation is not apparent from the data which we have in these cases. There was no definite correlation with mediastinal displacement: M. P. had marked displacement; E. S. when his pneumothorax was complete showed almost none.

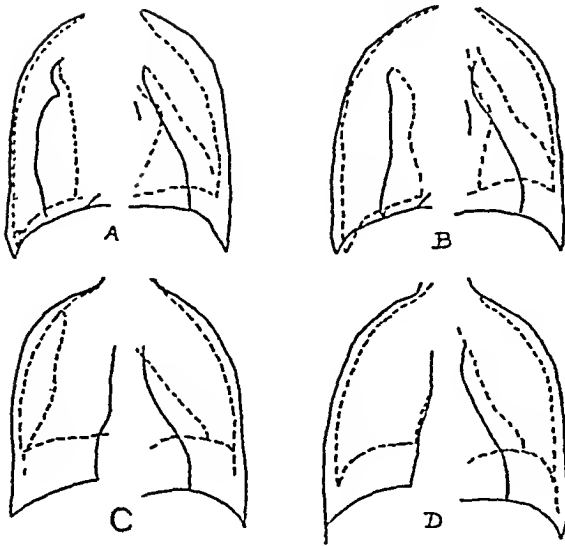


FIG. 3. X-RAY OUTLINES OF SUBJECT M. P.

Similar to Fig. 2. A, January 27th; B, January 31st; C, February 25th; D, March 9th, 1933.

(See Figures 2 and 3.) No consistent relation has been found with venous or pleural pressures, oxygen intake or chemical changes in the blood. Simple decrease in pulmonary vascular bed, as suggested by Richards, Riley and Hiscock, does not appear to be a determining factor. The only phenomenon observed that showed an apparent relation to decrease in cardiac output was the change in diaphragmatic movement produced by pneumothorax. The three cases whose cardiac output was decreased showed also either a greatly diminished or a paradoxical movement of the diaphragm on one side. The two cases of spontaneous pneumothorax and the fourth case (D. J.) of partial artificial pneumothorax had relatively little alteration in diaphragmatic excursion. Possibly pulmonary blood flow is related rather to the degree of ventilation of the collapsed lung than to the degree of collapse.

If one assembles all the cases thus far published in which the technique of measurement was probably adequate, one reaches the tentative inference that in simple unilateral pneumothorax there is a tendency to diminished cardiac output, which in individual cases may be marked, slight or absent. The situation may be not unlike the circulatory adaptation associated with change of posture in normal subjects, which shows similar differences in individual cases.

## SUMMARY

1. Measurements of cardiac output have been made in six cases in various stages of unilateral pneumothorax. The method employed the Fick principle, arterial blood being drawn for arterial  $\text{CO}_2$ , and oxygenated venous  $\text{CO}_2$  being obtained by a method described in the preceding paper.

2. In two cases of artificial pneumothorax cardiac output was definitely diminished when unilateral pneumothorax was established. In a third case there was a similar trend but not definitely beyond the error of the method. In one case of artificial pneumothorax, and in two cases of spontaneous pneumothorax with positive intrapleural pressures, no consistent relation between cardiac output and state of pneumothorax was demonstrated.

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#### PROTOCOLS

E. S., male, age 17, clerk, American. Admitted to Bellevue Hospital, September 4, 1931.

History—Tuberculosis found on routine x-ray. Family history: Sister had tuberculosis for five years.

Physical examination—Very well developed and well nourished. Few crackling râles between 2d and 3d interspaces on left.

X-ray—Few nodular shadows at right apex and left hilum, and possibly small cavity in latter region.

Course—Rest cure for 6 months. After acute upper respiratory infection x-ray showed increase in shadows on left with 3 cm. cavity. Sputum then positive. Pneumothorax started July 5, 1932. On August 6th developed fever and chest fluid, fever lasting until mid-September. Sputum negative after August 18th. Mediastinum displaced before fluid developed, but fixed in mid position after fluid was absorbed. Patient discharged December 9, 1932.

M. P., female, age 22, clerk, American. Admitted to Bellevue Hospital, September 9, 1932.

History—Following ether anesthesia, patient developed,

on August 11th, an abscess of the right upper lobe, characterized by severe sharp pain, followed by purulent foul sputum amounting to 2 oz. daily. At the time of admission the expectoration had increased to 4 oz.

Physical examination—Well nourished white female, not dyspneic; coughing moderately, expectoration about 4 oz., foul. Slight dulness over the right upper lobe posteriorly with very few coarse râles on coughing. No clubbing of the fingers. Rest of the examination negative.

X-ray—Small cavity at the right apex.

Sputum—Negative for tubercle bacilli.

Course—Patient put on postural drainage; bronchoscoped on October 5th; expectoration gradually decreased to about 4 cc. and became mucoid in character and odorless. Artificial pneumothorax started November 16th to collapse the small residual cavity. Small re-fills were given twice weekly. On January 31, 1933 the biggest collapse was obtained, about 60 per cent with moderate displacement of the heart under neutral pressure. From that time on the lung was allowed to reexpand, cavity having disappeared. Patient was discharged on March 25th, the lung having fully reexpanded at that time; had no sputum, no cough.

D. J., age 20, salesman, Irish. Admitted to Bellevue Hospital, March 14, 1932.

History—Onset 12 months previous to admission with progressive lassitude and slight cough. Small hemoptyses at the beginning of March; practically no expectoration. Past history irrelevant. Family history: father died from tuberculosis.

Physical examination—Very slight cough, productive of very small amount of greenish sputum. Few crackling râles at the right apex; very good motion of the diaphragm on both sides and expansion of both halves of the chest.

X-ray—Fibrotic change in the upper part of the right upper lobe with a cavity 3 cm. in diameter. Rest of the film normal.

Sputum—Positive for tubercle bacilli on admission.

Course—Fever for first week after admission, none thereafter. Right pneumothorax started April 29th; re-fills then given twice a week. Good lung collapse with little mediastinal displacement. Sputum negative on May 20th. Patient left the hospital June 5, 1932.

F. P., male, age 26, waiter, Italian. Admitted to Bellevue Hospital, March 10, 1932.

History—Onset 1½ years previous to admission with loss of weight, 35 pounds, slight cough, increasing in past 6 months; pain in left chest. Stopped working the first of March. Past history, negative. Family history, negative.

Physical examination—Slightly emaciated; moderate cough and expectoration. Signs of small cavity in

the left upper lobe; slight dulness, bronchial breathing and few crackling râles in the 1st and 2d interspaces anteriorly. Right lung apparently clear. Good motion of the diaphragm on both sides; symmetrical expansion of both halves of the chest. No dyspnea, no cyanosis.

X-ray—Showed a cavity, 4 cm. in diameter, located at the outer part of the left chest, at the 5th and 6th rib posteriorly and 2d and 3d rib anteriorly. Very slight nodular infiltration in the outer part of the right chest.

Sputum—Positive for tubercle bacilli.

Course—After 6 weeks of rest in bed, no improvement. Artificial pneumothorax was started on the left side on April 14th. Good collapse was obtained with progressive increase of pressure. Patient developed some fluid, almost from the start, which after May 10th did not show any tendency to increase. His mediastinum was displaced to the right with marked respiratory swing. Sputum negative on June 1st. Patient discharged August, 1932.

I. A., age 24, salesman, Puerto Rican. Admitted to Presbyterian Hospital, January 21, 1933.

History—Mild cough for 2 years. Chest x-ray 2 years previously was negative. Sudden onset of tightness in chest, 4 hours before admission, associated with dyspnea, pain in chest and palpitation.

Physical—Comfortable when at rest. Right side of chest immobile. Signs of large pneumothorax on right. Musical amphoric breath sounds heard over right chest. Temperature normal.

X-ray—90 per cent collapse of right lung. Considerable shift of mediastinum to left. Increased bronchial markings on left.

Sputum—Repeatedly negative. Vital capacity 1500 cc.

Venous pressure 80 mm. H<sub>2</sub>O. Pleural pressure + 8, + 10 cm. H<sub>2</sub>O.

Course—Slight reexpansion of lung in first week, then a second and even more complete collapse. Reexpansion proceeded steadily thereafter for the next 6 weeks, at which time it was practically complete and the patient was allowed to return home (March, 1933). He had been well except for recurrent upper respiratory infections, when heard from one year later.

L. P., age 29, salesman, Canadian. Admitted to Presbyterian Hospital, February 28, 1933.

History—Sudden onset of pain in right chest in February, 1933, followed by progressive dyspnea. Admitted to hospital 6 days later.

Physical examination—Right side of chest more expanded than left and immobile. Signs of large pneumothorax. Whistling amphoric breathing heard over right lower chest. Temperature normal.

X-ray—85 per cent collapse of right lung. Moderate shift of mediastinum to left. Linear fibrosis in left upper lobe.

Sputum—Repeatedly negative. Vital capacity 1900 cc. Venous pressure 105 mm. H<sub>2</sub>O.

Course—Improvement with reexpansion of lung for 1 month. Vital capacity 2900 cc. Venous pressure 45 mm. H<sub>2</sub>O. Collapse of lung 40 per cent. Then a second spontaneous pneumothorax occurred, collapse of lung 85 per cent, vital capacity 1950 cc., venous pressure 78 mm. H<sub>2</sub>O, pleural pressure + 3, + 10 cm. H<sub>2</sub>O. Condition unchanged for 2 weeks, after which re-expansion began and continued gradually. Discharged at the end of May with lung reexpansion nearly complete. Was well when heard from 4 months later.

# CYTOLOGIC STUDIES ON RHEUMATIC FEVER

## II. CELLS OF RHEUMATIC EXUDATES

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Previously (1), it was shown that rheumatic granulomata are typified by cells which have a characteristic appearance when stained supravitaly with neutral red and Janus green (2), and which differ, in their reaction to these dyes, from the typical cells of tuberculous and experimental syphilitic granulomata. The purpose of the present study was to investigate similarly the cells of rheumatic exudates, and to compare the cytologic characteristics with certain clinical features of the disease. It was further proposed to learn whether cells like those of rheumatic granulomata might be found in these exudates and thus serve as a diagnostic aid in questionable cases.

Forkner, Shands and Poston (3) and Forkner (4) have reviewed the work done upon the cytologic aspects of synovial fluid prior to the introduction of methods for supravital staining, and have indicated the uncertainty which exists concerning the nature of cells described in those studies because of the limitations of the methods used. Key (5) employed supravital staining in examining cells of synovial fluid of normal rabbits; and similar studies were made on synovial fluid of normal cattle by Bauer and his co-workers (6). Key (7) likewise has described the cells in experimentally induced arthritis of rabbits; and Forkner, Shands and Poston (3) and Shands (8), employed the same technique in studying the synovial fluid in human chronic arthritis. No reports of comparable investigations in rheumatic fever are available.

### MATERIAL AND TECHNIQUE

Sixty-two arthritic, 8 pleural and 5 pericardial exudates from 33 patients with rheumatic fever were compared with 35 similar exudates from patients with other diseases; 3 fluids from the joints of patients with cardiac edema and 2 from supposedly normal joints were also studied. (See Tables I and III for data on synovial exudates.)

Fluids were aspirated aseptically under novocaine anesthesia. All synovial exudates were obtained from knee joints prior to antirheumatic medication; and, in order to estimate the amount present, all obtainable fluid was removed.

Record was made of the total amount and character of the fluid, of the total number of white cells per c.mm., and of the differential counts made upon living and fixed cells. In some instances, the fluid was centrifuged and the sedimented masses of cells were fixed, imbedded in paraffin and examined in ordinary microscopic sections.

Fixed films of aspirated fluids were stained by several methods, the most satisfactory of which was a modification of the malachite green-acridine red stain of Hitchcock and Ehrich (9). As soon as dry, the films were fixed in Zenker-acetic solution for 2 minutes, washed in running water for 10 minutes, immersed in the mixed stain solution for about 30 seconds, rinsed very quickly first in water and then in absolute alcohol, and finally passed to xylol and balsam. With this method, the cells were never obscured by deeply colored debris as they often were in films stained with eosin-methylene blue and by Wright's and other methods.

The supravitally stained cells were examined in a warm-box at 37° C. within 30 minutes of aspiration. Drops of fluid were placed upon cover slips which were then inverted upon slides covered with a dry film of neutral red and Janus green. The details of the method are given elsewhere (1, 10).

It was possible to record the differential counts in three ways: (1) in terms of percentages of the various cell types, (2) as the number of each type per c.mm., or (3) as the approximate total number of each type contained within the individual joint cavities. The latter method was chiefly employed.

### RESULTS

#### *Cytologic changes in the fluid of a single joint during the course of rheumatic polyarthritis*

An attempt was made to follow the cytologic changes in the fluid of a single rheumatic joint as the inflammation progressed and receded. To this end, samples of synovial fluid were obtained, at intervals of one to four days, from the knee joints of two patients (P. G. and J. Si., Table I).

TABLE 1

Summary of data for synovial fluid from patients with rheumatic fever—only specimens from joints uncomplicated by previous aspiration are listed

Patient	Age	Sex	Joint	Duration		Severity of arthritis*	Stage of arthritis*	Temperature†	White blood count‡	Amount of fluid	Character of fluid	White cells per c. mm. synovial fluid	Polya.	Glamato-cytes	Lymphocytes	Mono-cytes	Undifferentiated cells§	Other cells
				of disease	of arthritis*													
	years			days	days			°F.		cc.			per cent	per cent	per cent	per cent	per cent	per cent
M. M.	23	M.	Knee	17	17	±	improving	100.8-103	11,000	?	?	14,860	49	31	15	2	3	0
J. O. B.	37	M.	Knee	10	5	+	improving	103-104	14,000	25	flaky	1,440	18	50	16	14	2	0
I.	47	M.	Rt. Kn.	21	6	+	improving	102-104	12,000	40	cloudy	10,020	85	11	2	0	2	0
			Lt. Kn.	21	6	+	improving	102-104	12,000	15	bl. tinged		12	80	0	2	6	0
C. M.	12	F.	Rt. Kn.	12	7	±	improving	100	6,000	5	clear	1,200	35	4	40	0	4	16
			Lt. Kn.	12	5	±	improving	100	6,000	20	clear	1,620	39	33	16	3	0	4
S. S.	10	M.	Knee	19	3	+++	advancing	103-105	31,000	10	bl. tinged	25,800	87	9	2	1	1	0
J. Sw.	18	F.	Knee	11	3	++	stationary	102-103	22,000	10	clear	23,600	83	14	3	0	0	0
J. Sk.	49	M.	Rt. Kn.	?	7	+	improving	99.6-103	13,000	15	flaky	3,000	54	27	8	1	0	10
			Lt. Kn.	?	5	+	improving	99.6-103	13,000	20	flaky	3,840	36	46	1	1	6	10
E. B.	8	M.	Knee	17	4	±	stationary	101-103	11,000	2	clear	not done	66	16	14	2	0	2
J. P.	27	F.	Rt. Kn.	45	7	++	advancing	101-103	8,000	8	flaky	5,400	40	33	14	0	8	0
			Lt. Kn.	47	16	+++	advancing	101-103	23,000	15	?	7,260	80	13	4	0	0	3
G.	6	M.	Knee	33	23	+	stationary	100-103	25,000	1	?	47,600	97	0	1	2	0	0
P. G.	20	M.	Rt. Kn.	14	11	±	improving		13,000	45	clear	800	10	7	82	0	0	1
			Lt. Kn.	14	14	±	improving		13,000	35	clear	1,040	14	14	65	4	2	0
T.	7	M.	Knee	13	5	±	improving	101-103	33,000	4	flaky	35,500	84	15	1	0	0	0
Sor.	15	M.	Rt. Kn.	12	12	+	improving	104-105	11,000	4	clear	2,800	75	6	11	0	5	3
			Lt. Kn.	72	72	±	stationary	99-100.5	9,000	0.1	flaky	Q.N.S.	8	0	90	0	0	2
Pop.	13	M.	Knee	28	6	±	improving	99-100	8,000	?	clear	5,200	70	17	2	9	2	0
Sor.	16	M.	Knee	6	3	+++	advancing			35	cloudy	17,550	95	0	0	2	2	0
Pop.	14	M.	Knee	10	10	+++	stationary	102.6	9,000	5	flaky	7,000	87	0	1	1	11	0
Frank.	21	M.	Knee	8	7	±	improving	101-102	15,000	5	flaky	1,640	2	16	33	2	2	40
Cl.	38	M.	Rt. Kn.	8	8	++	stationary		11,000	25	flaky	1,420	34	0	22	32	12	0
			Lt. Kn.	8	8	+++	stationary		11,000	45	flaky	1,000	48	24	0	14	14	0
H. D.	17	M.	Knee	12	6	+++	advancing	104.2	14,000	30	cloudy	7,400	95	0	0	5	0	0
El.	10	M.	Rt. Kn.	7	3	++	stationary		11,000	10	cloudy	40,000	91	6	2	1	0	0
			Lt. Kn.	7	5	++	advancing		11,000	15	cloudy	27,400	94	0	0	6	0	0
J. C.	33	M.	Rt. Kn.	17	17	+	improving	101	13,000	5	cloudy	4,200	5	83	3	9	0	0
			Lt. Kn.	17	4	+	improving	101	13,000	20	cloudy	6,500	13	77	1	9	0	0
S.	14	M.	Rt. Kn.	6	6	++++	stationary	103		35	cloudy	4,240	65	8	3	18	6	0
			Lt. Kn.	6	5	++++	stationary	103		30	cloudy	6,400	86	2	0	10	1	1
L. E.	15	F.	Knee	28	25	+	advancing	102	8,200	12	cloudy	11,000	89	3	3	2	3	0
Pet.	30	F.	Rt. Kn.	15	5	++	advancing	105	12,000	18	cloudy	10,400	89	0	3	8	0	0
			Lt. Kn.	15	5	++	advancing	105	12,000	20	flaky	7,400	93	7	0	0	0	0
Scl.	14	M.	Knee	?	7	++	improving	100.8	7,500	16	cloudy	23,600	60	30	0	0	10	0
F. McS.	11	M.	Knee	17	6	++	advancing	103.5	23,000	?	?	3,600	91	0	4	5	0	0
Weis.	33	M.	Knee	6	6	++	improving	103.4		10	cloudy	5,850	74	23	1	0	2	0
J. Sl.	20	M.	Rt. Kn.	8	6	+	advancing	100.4-101	16,000	2	clear	10,500	85	0	14	0	1	0
			Lt. Kn.	9	17	++	advancing	100.4-101	16,000	5	bl. tinged	22,000	89	1	0	1	0	0

\* of the particular joint aspirated

† figures show temperature at time of aspiration or give range during 24 hours before and after aspiration.

‡ on day of aspiration

§ designates undifferentiated young connective tissue cells

|| includes degenerated and unclassified cells

*Total white cell content of rheumatic synovial exudates*

The total number of white cells per c.mm. of synovial fluid varied between 800 and 47,600 with an average of 10,750. The frequency distribution is shown in Table II. The total number of

TABLE II

*Distribution of total white cell counts obtained in thirty-seven exudates from rheumatic knee joints uncomplicated by previous aspiration*

Total white cells per c.mm.	Number of exudates
Under 1000 .....	1
1000 to 5000 .....	13
5000 " 10000 .....	9
10000 " 15000 .....	5
15000 " 20000 .....	1
20000 " 25000 .....	3
25000 " 30000 .....	2
30000 " 35000 .....	0
35000 " 40000 .....	1
Above 40000 .....	2

white cells contained within individual knee joints was estimated to vary between 60,000 and 6,143,000 with an average of 1,447,000.

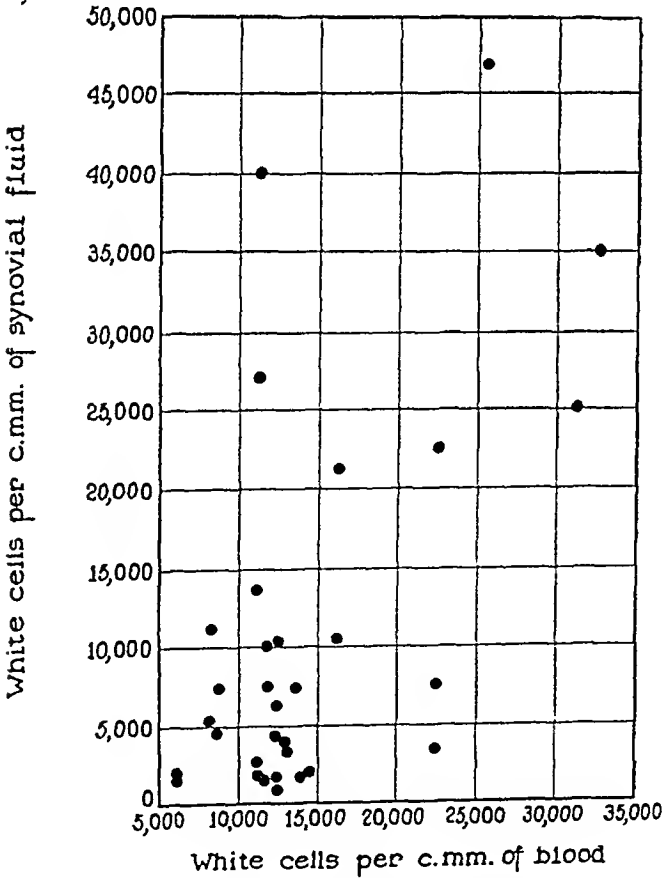


FIG. 3. COMPARISON OF NUMBER OF WHITE CELLS PER C.MM. OF BLOOD AND SYNOVIAL FLUID OF INDIVIDUAL PATIENTS.

In the same patients, the white cells in the blood varied between 6,000 and 33,000 with an average of 15,800 per c.mm., each blood count having been done on the same day as the corresponding count on synovial fluid. Figure 3, in which these two features are compared, suggests, perhaps, a slight tendency for them to vary directly.

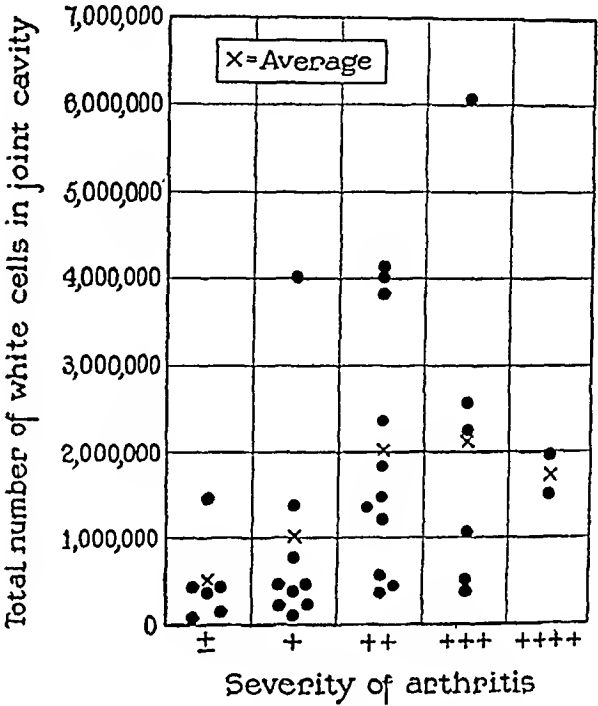


FIG. 4. RELATION OF ESTIMATED TOTAL NUMBER OF WHITE CELLS IN JOINT CAVITY TO SEVERITY OF ARTHRITIS.

Figure 4 indicates the relationship between the estimated total white cell content of the synovial fluids and the degree of inflammation of the individual joints. While some of those more severely involved contained relatively few cells, there was a slight correlation. This tendency was even less apparent when the number of cells per c.mm. was considered instead of the total cellular content.

In Figure 5 is shown the relation between the total number of white cells estimated to have been in the joint cavities and the stage of inflammation in those joints. While no exact correspondence between the two was found, the averages indicate a tendency for increasing arthritis to be accompanied by higher cellular content per joint.

Figure 6 records the relationship between the total number of white cells within individual joint cavities and the duration and stage of arthritis in the same joints. Involvement under 7 days tended to be associated with higher counts, but

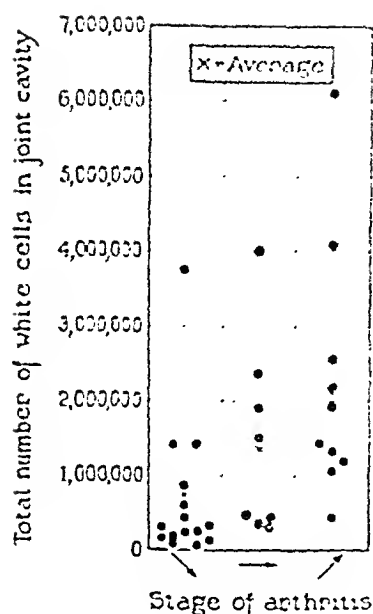


FIG. 5. RELATION OF ESTIMATED TOTAL NUMBER OF WHITE CELLS IN JOINT CAVITY TO STAGE OF ARTHRITIS.

\ = arthritis improving.  
 — = " stationary.  
 / = " becoming worse.

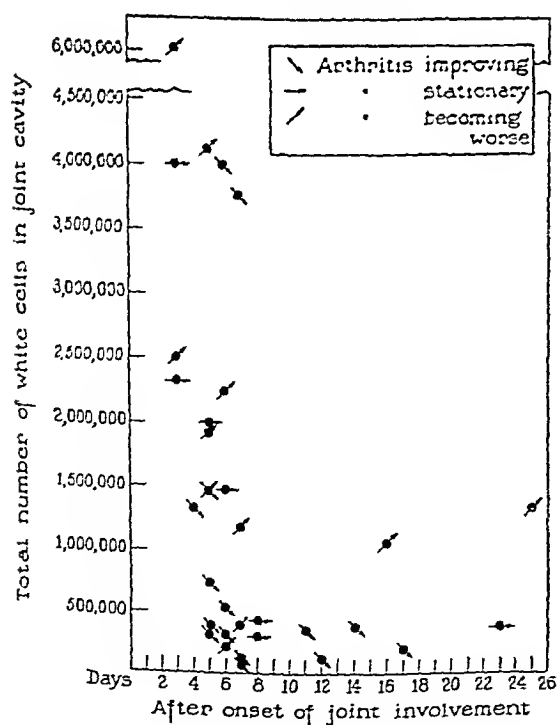


FIG. 6. ESTIMATED TOTAL WHITE CELL CONTENT OF JOINTS COMPARED WITH DURATION AND STAGE OF ARTHRITIS.

in the small number of joints aspirated for the first time after more prolonged arthritis, the relationship was vague. As was to be expected, the stage of arthritis in the individual joints had more bearing upon the total cellular content than had the duration, and it should be noted that the relatively high counts obtained after 16 and 25 days of arthritis were from joints which had suffered relapses and were increasing in severity of involvement at the time of aspiration.

#### *Differential formulas of white cells in rheumatic synovial exudates*

In the following discussion, the large mononuclear cells have been divided into monocytes and clasmatocytes according to the classification of Sabin, Doan and Cunningham (11).

Fixed, stained films of synovial fluid from patients with rheumatic fever showed large mononuclear cells, polymorphonuclear leukocytes in various stages of degeneration, and lymphocytes. Some of the large mononuclear cells were so filled with vacuoles and phagocytosed material as to leave little doubt that they were clasmatocytes; the majority, on the other hand, could not be accurately classified in the fixed preparations.

Cells of the type shown in Figure 7 were encountered frequently and, at first, proved difficult to classify. When stained by Wright's method

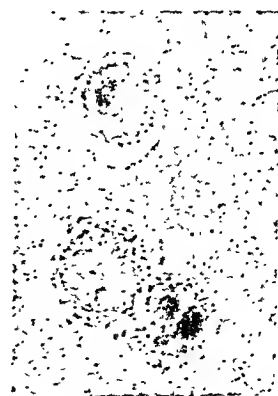


FIG. 7. MICROPHOTOGRAPH OF POLYMORPHONUCLEAR NEUTROPHILES IN SYNOVIAL FLUID.

Middle cell is normal; that at right shows nucleus becoming rounded-up and compact; that above shows more advanced degenerative changes with a single nuclear mass. The apparent granules in the two degenerated cells are artifacts caused by deposit of coagulated material from the synovial fluid. Wright's stain.  $\times 1000$ .

TABLE III  
Summary of results of cell counts on synovial fluids

Diagnosis	Num- ber of fluids	White cells per c. mm.			Polynuclear neutrophils			Clasmatocytes			Monocytes			Lymphocytes			Undif. cells*			Indeterminate macrophages			Synovial cells		
		Min- imum	Max- imum	Aver- age	Min- imum	Max- imum	Aver- age	Min- imum	Max- imum	Aver- age	Min- imum	Max- imum	Aver- age	Min- imum	Max- imum	Aver- age	Min- imum	Max- imum	Aver- age	Min- imum	Max- imum	Aver- age	Min- imum	Max- imum	Aver- age
Normal	2	125	200	160	7	27	17	5	8	6	5	16	10	0	16	8	12	16	14	0	13	6	3	7	5
Rheumatoid Arthritis	8	3,640	17,300	10,500	9	94	66	0	9	3	0	22	7	3	56	21	1	4	2	0	0	0	0	0	0
Gonococcal Arthritis	3	7,950	15,750	11,100	72	93	85	1	6	3	0	1	.7	3	18	10	0	3	1	0	0	0	0	0	0
Syphilitic (Infectious) Arthritis	3	14,800	42,800	24,700	6	93	58	0	8	3	0	18	6	7	57	25	9	13	10	0	0	0	0	0	0
Tuberculous Arthritis	2	6,000	7,050	6,500	0	17	8	0	2	1	20	26	23	61	66	63	0	4	2	0	0	0	0	4	2
Infectious Arthritis of Un- certain Type	5	9,800	58,750	27,500	72	94	83	0	11	5	0	15	6	0	9	4	0	5	2	0	0	0	0	0	0
Pyogenic (Hemolytic Strep.) Arthritis	2	20,000	Too many to count	?	97	100	98.5	0	2	1	0	0	0	0	0	0	0	1	.5	0	0	0	0	0	0
Traumatic Arthritis	2	?	?	?	37	67	52	0	6	3	3	5	4	24	58	41	0	0	0	0	0	0	0	0	0
Rheumatic Fever	40	800	47,600	10,750	2	98	66	0	83	16	0	32	4	0	90	12	0	14	2	0	0	0	0	0	0

\* This refers to undifferentiated young connective tissue cells.

they were round and of the size of small or intermediate lymphocytes; the pink-stained cytoplasm presented a faintly stippled appearance but no granules; and the dark blue nuclei were round and pyknotic. When stained with malachite green and acridine red (9) they showed almost colorless cytoplasm and dark green nuclei. Their nature became clear when further study revealed all gradations between them and normal polymorphonuclear neutrophils (Figure 7); and comparison of fixed films and supravital stained preparations confirmed the belief that they were merely rounded-up, degenerated neutrophils. It is probable that they have been mistaken for nucleated red cells, and their presence in exudate from a traumatized joint might lead, therefore, to an erroneous diagnosis of intracapsular fracture (12).

With supravital staining it was possible to classify most of the indefinite group of large mononuclear cells of the fixed preparations, into clasmatocytes and monocytes. The characteristics of all types of cells of synovial fluid have been amply described by Key (5, 7, 15), Forkner, Shands and Poston (3), Bauer and his co-workers (6), and others; and hence they will not be discussed here. In addition to polymorphonuclear neutrophils, lymphocytes, clasmatocytes and monocytes there was a moderate number of cells of the same general character as the latter but smaller and containing only a few small, scattered neutral red bodies and few or no mitochondria. These were interpreted by Dr. Florence R. Sabin as undifferentiated young connective tissue cells beginning to develop into either monocytes or clasmatocytes but not yet having progressed far enough to permit of definite identification. The minimum, maximum and average figures for all these cells in a series of 40 exudates from rheumatic knee joints are shown on the bottom line of Table III. Four of the specimens contained, in addition, 1 to 2 per cent of eosinophiles. The indeterminate macrophages of Key (5) were not found in fluids from inflamed joints. In every sample occasional erythrocytes were encountered, but, whenever numerous, their presence was attributed to trauma of aspiration.

Small masses of cells were numerous in all flaky fluids and, in every instance in which identification was possible, the cells composing them were clasmatocytes and polymorphonuclear leuko-

cytes. In two fluids, small masses were seen which were perhaps composed of synovial lining cells, although the clumps were too thick for exact identification of individual elements. In none of the samples of synovial fluid from inflamed joints were isolated synovial lining cells identified.

An important relationship appeared to exist between the differential formulas and (1) the stage of arthritis, and (2) the age of the patient. In Figure 8 are plotted the differential counts for the individual joints in which arthritis was becoming worse, remaining unchanged, and improving; and these data, as well as the averages, are recorded separately for patients under and over twenty years of age. The age of twenty was chosen arbitrarily as the dividing line between the younger and older groups. Although the number of specimens in each category is too small for statistical analysis, and the spread of percentages in several of the categories is considerable; nevertheless, the trends shown by the distribution of individual counts and the averages for the various categories appear definite enough to be significant. With increasing arthritis there was a great predominance of polymorphonuclear neutrophils and few clasmatocytes. With improvement, on the other hand, the percentage of clasmatocytes increased. Furthermore, the specimens from patients in the younger age group contained a higher ratio of polymorphonuclears to clasmatocytes than did those from older patients. Thus, the age of the patient and stage of arthritis appeared to have sufficient relationship to the cellular reaction in the synovial exudate to make difficult the interpretation of findings in any given specimen unless these two factors were taken into consideration. For example, the polymorphonuclear neutrophils in synovial fluids from patients under twenty years of age whose arthritis was increasing, averaged 92 per cent of the white cells present; while in the fluids from improving joints of older patients, the polymorphonuclears averaged only 31 per cent, but the clasmatocytes 39 per cent. The figures for the lymphocytes tended to parallel those for the clasmatocytes. On the other hand, no such correlation was evidenced by the monocytes and undifferentiated young connective tissue cells, which were present in all fluids in approximately equal numbers. While the cytologic differences in the three stages of arthritis were not



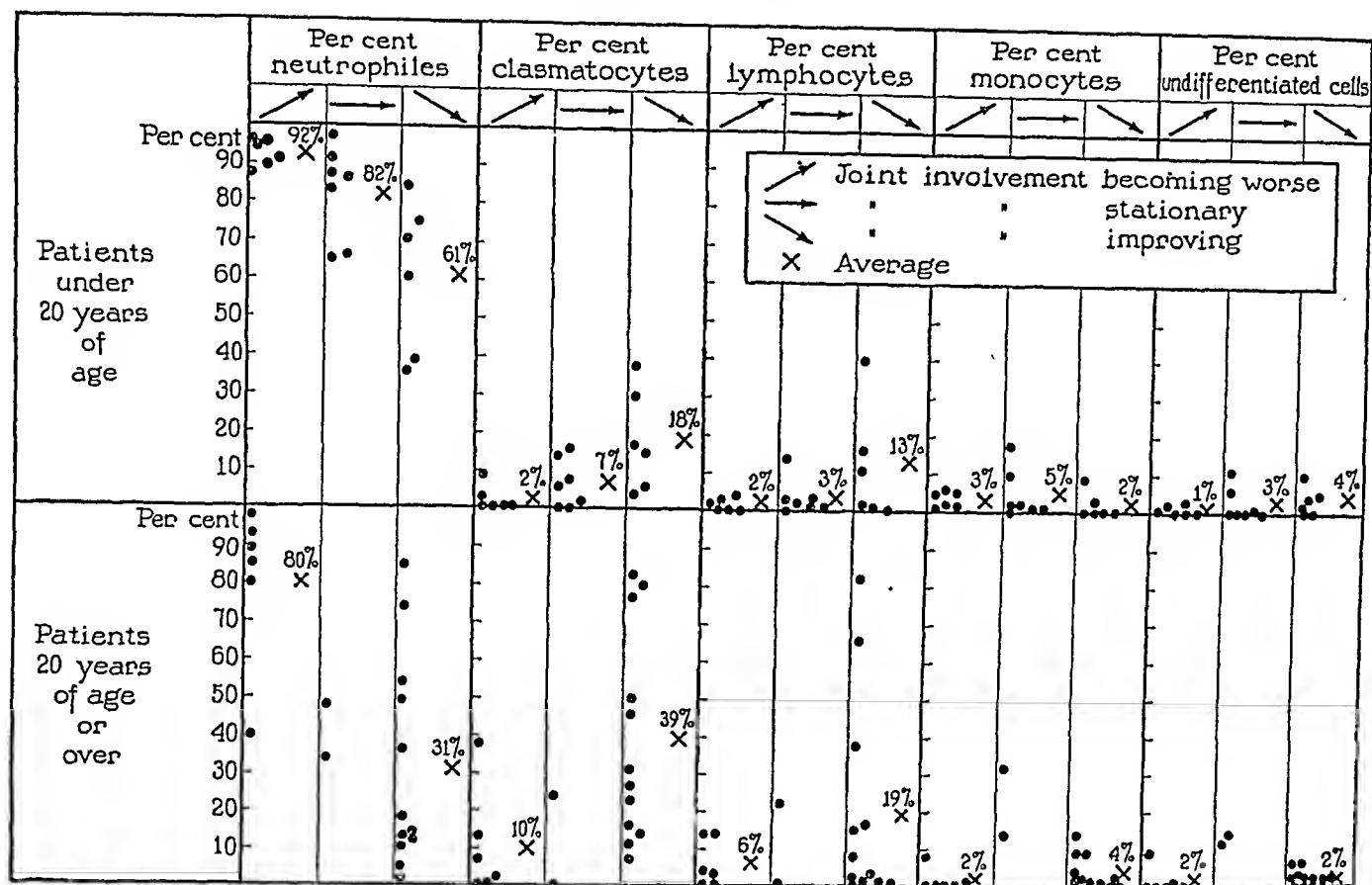


FIG. 8. CORRELATION BETWEEN STAGE OF ARTHRITIS AND DIFFERENTIAL FORMULAS OF WHITE CELLS IN SYNOVIAL FLUID FROM YOUNGER AND OLDER PATIENTS.

Each dot represents the finding for a given specimen of fluid.

surprising, there could be found no explanation for the apparent differences in the two age groups. One wonders, however, whether the latter may not be in some way related to the well known clinical differences often noted in rheumatic polyarthritis as it occurs in these two age groups.

The duration and severity of arthritis apparently had little bearing upon the type of cell predominant in the fluid other than that more of the early and severely involved joints tended to fall in the group showing an increasing severity of arthritis.

#### *Pleural and pericardial exudates from patients with rheumatic fever*

The findings in the 8 pleural and 5 pericardial exudates were essentially the same as those in synovial fluid. The total amounts of fluids were unknown, but in appearance they varied from clear to flaky. The total number of white cells varied from 1,600 to 12,400 per c.mm. with polymorphonuclear neutrophils from 4 to 67 per cent, lymphocytes from 0 to 74 per cent, mono-

cytes from 0 to 6 per cent, clasmatocytes from 4 to 74 per cent, undifferentiated young connective tissue cells from 0 to 7 per cent, and eosinophils from 0 to 4 per cent. The most striking cytologic difference between these exudates and those from joints was the presence of 2 to 14 per cent of desquamated mesothelial cells in every sample. These cells, the characteristics of which in supravital stained preparations have been described by others (3, 11), occurred singly or in groups of two to four, and were easily distinguished from all other types.

#### *Synovial fluid from non-inflamed human knee joints*

For comparison, it was desired to ascertain the number and types of cells present in the fluid of non-inflamed human joints. Samples were obtained from two normal knee joints and from three in which the only apparent abnormality was the presence of edema fluid. The first supposedly normal fluid was from a patient dying of cerebral hemorrhage, aspiration having been performed

immediately postmortem. Approximately 0.5 cc. of clear, very mucoid fluid was obtained which contained 125 white cells per c.mm. as well as occasional erythrocytes. In supravitaly stained preparations the differential count of 100 cells was as follows: polymorphonuclear neutrophils, 27 per cent; mature monocytes, 7; stimulated monocytes, 9; stimulated clasmatocytes, 8; undifferentiated young connective tissue cells, 12; synovial lining cells, 3; unidentified degenerated cells, 21; and indeterminate phagocytic cells, 13. The latter, which have been described by Key (5), were obviously mature elements containing neutral red bodies of varying size and sometimes mitochondria; they could not be classified into monocytes and clasmatocytes, however, as they had characteristics of both. The other normal fluid was obtained from the hip joint of a six year old girl at operation for congenital dislocation of the hip. An approximate total count of 200 white cells per c.mm. was obtained with the following differential picture: polymorphonuclear neutrophils, 7 per cent; monocytes, 5; stimulated clasmatocytes, 5; undifferentiated young connective tissue cells, 16; lymphocytes, 16; synovial lining cells, 7; and unidentified degenerated cells, 44.

In general these findings are in accord with those of Key (5) and Bauer (6) for the synovial fluid of normal rabbits and cattle; however, in both these specimens the monocytes were fewer than was the case with the animals, and in the first case the polymorphonuclear neutrophils were unexpectedly numerous. The large number of degenerated cells perhaps accounts for the comparatively few monocytes found, while Key's observation (5) that polymorphonuclear cells migrate into joint cavities shortly after death may explain the relatively numerous neutrophils in Case 1. This count was done very soon after death, but the patient had been moribund for some hours before, and it is not unlikely that a migration of polymorphonuclears may have started.

The presence of synovial lining cells in these fluids in numbers comparable to those of normal rabbits and cattle was in contrast to their apparent absence from the arthritic exudates. Perhaps the small number of them normally desquamated in the latter were lost sight of in the great number of inflammatory cells. In the normal fluids they had the appearance described by Key (5, 15),

Bauer and his co-workers (6), and Forkner, Shands and Poston (3); granules or vacuoles staining with neutral red were never seen either in these specimens or in scrapings of normal human and rabbit synovial membranes.

Of the specimens from patients with non-rheumatic cardiac edema, one was aspirated during life and the other two immediately postmortem. The amounts obtained were 22 cc., 3 cc. and 8 cc. respectively. The white cells were so few that total counts were unsatisfactory and differential counts incomplete. In the first sample only two cells were seen after prolonged search: a small lymphocyte and a stimulated clasmatocyte. In the second, in addition to 9 erythrocytes, five cells were identified: two small lymphocytes, two stimulated monocytes, and a phagocytic clasmatocyte. In the third specimen, there were: one erythrocyte, two unstimulated monocytes, and an indeterminate phagocytic cell. Taking into account the dilution by edema fluid, these figures are probably comparable to those reported for normal rabbits and cattle.

While no definite conclusions regarding the cellular content of non-inflammatory human synovial fluid can be drawn from so few samples, the results obtained do serve as a rough base line for observations upon the fluids from inflamed human joints.

#### *Joint exudates from patients with diseases other than rheumatic fever*

As controls, 25 exudates from joints of patients with diseases other than rheumatic fever were examined. Among these were: rheumatoid arthritis,<sup>2</sup> 8; gonococcal arthritis, 3; syphilitic arthritis (infectious), 3; tuberculous arthritis, 2; purulent (hemolytic streptococcal) arthritis, 2; infectious arthritis of uncertain type, 5; and traumatic arthritis, 2. The results obtained are shown in Table III. The pus from the patients with purulent arthritis was, of course, unmistakable; in none of the other samples, however, was there any gross or cytologic characteristic which served to distinguish it from rheumatic exudates. In Shands' (8) study of synovial fluid from joints of patients

<sup>2</sup> All these patients had arthritis of the type characterized by fusiform swelling of the proximal interphalangeal joints and ulnar deviation of the fingers.

with infectious arthritis, the average percentages of lymphocytes and monocytes were higher than those obtained in the rheumatic exudates of the present series. On the other hand, individual synovial exudates of this series gave percentages of monocytes and lymphocytes considerably higher than the averages reported by Shands; so that cytologic examination of the synovial fluid is probably of little value in the differential diagnosis of individual cases.

The work of Sabin (13) and others would lead one to expect epithelioid cells and numerous monocytes in tuberculous arthritic exudates, and Geiger (14) has shown this to be the case in joint fluids from rabbits with experimental tuberculous arthritis. Contrary to expectation, however, the synovial exudates from two proven cases of early tuberculous arthritis, showed no epithelioid cells; and the monocytes, although more numerous than in the non-tuberculous exudates, had the customary appearance. In one other specimen, from a far advanced and caseous joint (not included in Table III), there were many degenerated polymorphonuclear neutrophils and clasmotocytes but no epithelioid cells or monocytes. These results do not imply, of course, that epithelioid cells are not often present in exudates from tuberculous joints. Work is in progress to study this further.

#### DISCUSSION

Although it was not possible to follow the cytologic changes during the course of rheumatic arthritis in a single joint, the picture can, perhaps, be reconstructed from the results reported in the foregoing study. The primary response seemed to consist chiefly of polymorphonuclear neutrophils with a few monocytes and undifferentiated young connective tissue cells. As recovery began, clasmotocytes increased in number and disposed of fibrin and degenerating cells by phagocytosis. In one patient with typical rheumatic fever (Sor., Table I), a small amount of fluid remained in one knee joint 72 days, and the synovial exudate, after this interval, contained 90 per cent of lymphocytes. This perhaps indicates that lymphocytes are the predominant cells in rare rheumatic exudates of such long duration, although Table I also shows that lymphocytes were occasionally numerous fairly early in the disease. The changes thus outlined are similar in many respects

to those observed by Key (7) in synovial exudates of rabbits following injection of mild irritants into their joints. Such differences as exist may be due to the greater complexity of factors acting in rheumatic inflammation, as, for example, the tendency to relapse.

Finally, it is of interest to compare the cells of rheumatic granulomata and exudates. It had been suspected that exudates from patients with rheumatic fever might contain the characteristic cells of subcutaneous rheumatic nodules (1) and thus aid in the differential diagnosis of arthritis of uncertain type. This proved not to be the case, however, for no cells resembling those of the granulomata were seen in any exudate. In a previous study (1) it was concluded that the granuloma cells arise from undifferentiated connective tissue elements called by Maximow (16) primitive mesenchymal cells. Since the exudates likewise contained what were thought to be undifferentiated young connective tissue cells, a question arises concerning the relationship between them. In appearance they were entirely different: the granuloma cells showed neither mitochondria nor neutral red bodies and presented basophilic cytoplasm and very distinct cell membranes; the exudate cells, on the other hand, showed a general resemblance to young monocytes but with atypical and scanty mitochondria and neutral red bodies. Probably both types are closely related in origin, and it is suggested that their dissimilarities may be due to environmental differences and to continued development as fixed tissue elements in the one case but as wandering cells in the other. Certainly, the undifferentiated young connective tissue cells in the exudates here studied were in no way characteristic of rheumatic fever since they occurred equally in exudates from patients with other diseases. These observations are in keeping with Swift's (17) division of rheumatic manifestations into those chiefly proliferative and those primarily exudative, and coincide with the known pathologic changes of rheumatic fever; for numerous histologic studies have demonstrated that the proliferative lesions, as typified in the submiliary myocardial nodules and subcutaneous nodules, are composed of characteristic cells, but that the exudative lesions of joints, pleura and pericardium are, for the most part, cytologically non-specific.

## SUMMARY

The amount, character and cellular content of exudates from patients with rheumatic fever were studied in relation to certain clinical aspects of the disease and were compared with those of non-rheumatic exudates.

There was no obvious correlation between the amount of synovial fluid and the severity of arthritis; the number of cells per cubic millimeter ranged between 800 and 47,000 and the total number contained in the exudates tended to vary directly with the stage and severity of arthritis; the differential formula appeared to bear some direct relationship to the stage of arthritis and the age of the patient. Supravital stains revealed no cells similar to those previously described in rheumatic granulomata. Early there was a predominance of polymorphonuclear neutrophils, with a few monocytes and undifferentiated young connective tissue cells; later there were numerous clasmatocytes containing debris and degenerating cells. Rheumatic pleural and pericardial exudates contained cells similar to those of the joints but with the addition of a few mesothelial elements. Because non-rheumatic exudates were similar in microscopic content, no specific character could be assigned to exudates in rheumatic fever.

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# THE EXCHANGES OF WATER, ELECTROLYTES AND HEAT DURING PHENYLETHYLHYDANTOIN SICKNESS<sup>1</sup>

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Phenylethylhydantoin (nirvanol) has been used since 1919 in the treatment of chorea minor. Its continued ingestion gives rise to a reaction, probably of an allergic nature, which has been described by Ray and Cunningham (1) as follows: "The sedative effects of nirvanol usually appear on the second or third day of its administration. Fever develops on the seventh day, reaches a maximum on the ninth day, and subsides on the eleventh day. On the ninth day, a few reddish-brown maculo-papules appear on the thighs, wrists, or abdomen, the face often appears swollen and flushed, and a pale pink exanthem may appear on the soft palate or buccal mucosa. By the next day or two, the exanthem has become general. It is usually distinctly morbilliform, but somewhat more like urticaria than the rash of measles. Leukopenia was observed in eight of nine cases. A marked eosinophilia was observed in all specimens of blood that were studied during the eruption."

Nirvanol sickness bears a remarkable resemblance to serum sickness. Rackemann, Longcope and Peters (2) studied the exchange of water and chlorides in pneumonia patients who had received horse serum, and they observed a marked transient retention of water and chlorides. Other investigators (3) have at various times observed a similar retention of these substances in febrile diseases, notably lobar pneumonia. The present problem was undertaken in the hope that a controlled study of nirvanol sickness might indicate how general are the changes in metabolism during fever and allergic states.

## METHOD OF STUDY

Patients were maintained in the metabolism ward at the Strong Memorial Hospital, under the

care of specially trained nurses. The patient's day was divided into four periods of six hours each, beginning at 6 a.m. At the beginning of each period, the patient voided into a weighed bottle, and was then himself weighed on a balance sensitive to 5 grams. After this, he received a meal of exactly known weight and composition. The diet was entirely liquid, and was made up of distilled water, powdered milk, lactose, cream, eggs, and a small amount of added sodium chloride. In addition, the patient received daily a constant amount of orange juice and distilled water. Urine was divided into twelve-hour specimens, beginning at 6 a.m. Stools were collected in weighed containers. Temperature, pulse, respiration, barometric pressure, and humidity were recorded four times daily.

Complete water balances for each twelve-hour period were estimated by the method of Newburgh, Johnston and Falcon-Lesses (4). Water available to the body included water drunk, water in food, water formed by the oxidation of food, and water liberated by the catabolism of body tissue. Water lost included water of the urine, water of stools, insensible perspiration, and water held by stored food or tissues. Urine samples were wet-ashed, and pooled; stool specimens were dry-ashed. In the urine samples, total nitrogen was determined by the macro-Kjeldhal method, chlorides by the Volhard-Harvey method; sodium was determined gravimetrically as sodium-zinc-uranyl-acetate, and potassium by the method of Shohl and Bennett (5). The diet was checked for constancy of composition by daily determination of chlorides. The chloride content of the diet was found from day to day to show an average deviation of 0.83 per cent. The above determinations were made on four patients, each over a period of about three weeks, during part of which they received nirvanol. On a fifth patient, a complete acid-base balance of the urine was determined. This involved determination of

<sup>1</sup> The expenses of this investigation were defrayed in part by a grant from the Fluid Research Fund of the Rockefeller Foundation.

pH (electrometrically by means of a quinhydrone electrode), chlorides, phosphates, sulfates, ammonia, titratable acidity, and total inorganic base. Total inorganic base was determined by the method of Wright and Allison (6). All the patients were maintained on the diet for several days before nirvanol was started, and remained in the metabolism ward on the constant routine for several days after the reaction had subsided.

#### *Data on a representative case*

Of the first four cases, Case 4 was the most satisfactory. The diet was taken perfectly, and collections were perfect. Also, the patient developed a perfectly typical rash and fever. The findings

in the first three cases substantiated those of Case 4, although the reactions were less severe and the changes observed less pronounced. This patient was a ten year old girl with a previous history of tonsillitis and rheumatic fever; the findings are shown graphically in Figure 1. In interpreting these graphs, it should be remembered that the intake was exactly the same for each twelve hour period; the stools were few in number, formed, practically constant in composition, and must of necessity be neglected in considering the sudden changes in water and electrolyte balance.

Nirvanol, 0.5 gram, was given at noon each day for seven days. Time is plotted beginning with the first day of its administration. Fever

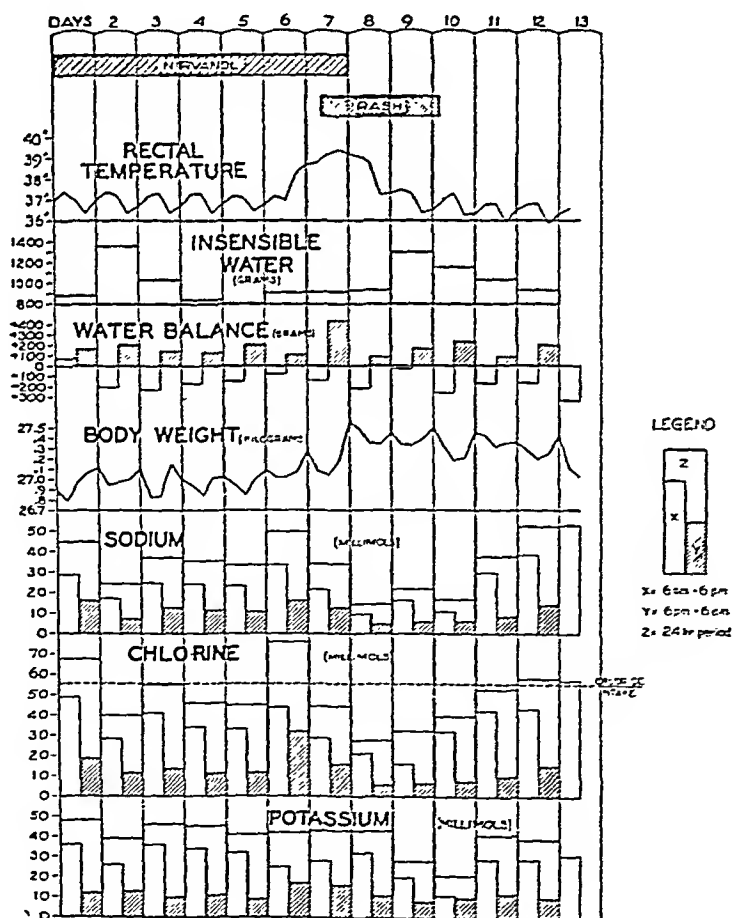


FIG. 1. SUBJECT M. C.

Shaded areas represent the night period. Insensible loss of water is plotted in 24 hour periods, water balance in 12 hour periods. Sodium, chlorine, and potassium are plotted to show day, night, and total 24 hour urinary output in milliequivalents.

began on the sixth day, and rash on the seventh, as indicated. The insensible loss of water became fairly constant after the first two days, and remained at the same level throughout the fever, showing no significant change until the first postfebrile day, during which there was a pronounced rise. The insensible loss then gradually returned to normal. During this postfebrile period, there was a noticeable tendency for the child to perspire visibly, but moderately. The water balance is the algebraic sum of the water available and the water lost. The water available remained constant within narrow limits throughout the experiment, so that any changes in the water balance are due to changes in the amount of water lost, chiefly through the kidneys.

It will be noted that there was a normal diurnal rhythm in the water balance, as has often been found. During the night period, represented by the shaded areas, there was normally a retention of between one and two hundred grams of water, and a corresponding loss during the day. Changes in water balance were closely paralleled by changes in body weight. A sudden retention of water occurred during the night period of the seventh day, the total water balance for this period being plus 450 grams, and the gain in body weight being 500 grams. This period of retention corresponded to the height of the fever and the beginning of the rash. Subsequently, there was no sharp loss of the retained water, but the weight showed a tendency to return to normal at the end of the experiment. In other cases also there was a similar sudden rise in weight with a gradual return to the normal. The total water available for twelve days amounted to 33,882 grams, and the total water lost was 33,748 grams, making a net balance for twelve days of plus 134 grams. The gain of body weight was 160 grams.

The outputs of sodium and of chloride ran closely parallel. They were excreted at a fairly constant rate on the third, fourth, and fifth days. The sudden increase in the output of these elements during the day fever began was observed in only one other case. A definite retention of sodium and chloride occurred on the eighth, ninth, and tenth days. This was interesting, because the retention of water all took place on the seventh day. Hence on the seventh day one must assume

that there was at least a slight dilution of the body fluids. The retention of these two elements on days eight, nine, and ten, was far in excess of the loss which occurred on day six.

The output of potassium was remarkably constant for the first eight days. On the ninth and tenth days, there occurred a marked retention. Grouped stool specimens from the period including these days of retention actually contained less potassium than the stools of the normal period. The time relations were again interesting. Water retention occurred on the seventh day, sodium retention began on the eighth day, and potassium retention began on the ninth day. In the other cases studied, the changes in the output of potassium were not nearly so striking, these cases having less severe reactions. Such a retention of potassium is unusual. The fact that the other cases showed only slight retention suggests the possibility of error in the analyses. However, the determinations were checked and rechecked, as many as six determinations being made on the same sample, and no error could be found. It might here be mentioned that throughout the experiments, the patients remained in nitrogen balance, and showed no significant variation in the nitrogen output, indicating that no errors were made in feedings and collections, also that there was no increase in protein catabolism.

According to Gamble et al. (7), knowing the amount of retained sodium and potassium, and their concentrations in the fluids of the body, one can calculate the amount of water that would have to be retained along with the minerals to maintain the isotonicity of the body fluids. Gamble divides the fluid of the body into two portions, one within cell membranes, the other outside cell membranes. The concentration of sodium in extracellular fluids is approximately 148 millimolar; the concentration of potassium in intracellular water is about 112 millimolar. In extracellular water, K equals about 0.017 Na (in terms of milliequivalents), and in intracellular water, Na equals about 0.425 K. Let Na equal the milliequivalents of sodium retained, and K the milliequivalents of potassium retained by the body. Then the following formulae may be applied: 
$$\frac{\text{Na} - 0.425 \text{ K}}{148}$$
 = liters of extracellular water needed, and



$\frac{K - 0.017 Na}{112}$  = liters of intracellular water needed.

The results in Case 4 have been treated as follows: the average urinary output of sodium and potassium for the first five days was considered to be the normal rate of excretion, corresponding to the fixed intake. The next five days included the period of the drug reaction, and any diminution in the output of minerals has been interpreted as a retention of these minerals within the body. Normal water balance for the ten-day period was assumed to be zero, and any deviation from this was considered to be water retained or lost from the body. The results of calculations are summarized in Table I. The agreement between the

TABLE I

*Summary of calculations on Case 4*

All figures for Na and K are in milliequivalents.

Period	Average output of Na per day	Average output of K per day	Retained Na	Retained K	Remarks
	<i>m. Eq.</i>	<i>m. Eq.</i>			
1st 5 days	35.0	43.6	0	0	Normal period
2nd 5 days	27.2	35.4	39.0	41.0	Period of reaction

Water needed for retained Na ..... 146 cc.

Water needed for retained K ..... 357 cc.

Total water needed ..... 503 cc.

Water balance for first ten days ..... 502 grams

theoretical amount of water necessary to maintain isotonicity of the body fluids and the amount actually retained is obviously better than can be expected from the method, and no doubt is accidental. However, it can be said in all fairness, that the figures given are evidence that sodium and potassium were retained in approximately the right amounts to compensate for the dilution of body fluids which apparently took place on the seventh day. In the figures used for calculations, sodium within muscle cells was taken to be equal to 0.425 times the potassium concentration. This is in all probability in error, since the figures were obtained from analyses of whole muscles, and it is likely that much of the sodium found was outside the muscle fiber in the surrounding extracellular fluid.

The mechanism whereby these shifts in water and mineral balance take place during febrile conditions is doubtless very complex. The kidneys probably play an important part. The following hypothesis may be suggested: as a result of the fever or of the action of metabolic products, the circulation to the kidneys is reduced and the kidney threshold for water is raised. The immediate effect of this is to dilute first the blood plasma, and later the intracellular fluids, thus causing a compensatory retention of sodium followed by a retention of potassium. The retention of potassium would be evident only in fevers not accompanied by destruction of tissue proteins and consequent liberation of potassium. The work of Fremont-Smith, Dailey and Thomas (8) has shown clearly that in fever, there is a dilution, both of the blood and the cerebrospinal fluid. This dilution is manifested by changes in specific gravity, total solids, chlorides, protein, and freezing point.

#### *Water balance data on other cases*

Figure 2 shows the results obtained in Case 3. This boy, aged 10, did not develop such a severe reaction as the patient just discussed, and consequently did not show such marked changes in water and mineral metabolism. The chart does show that such changes as did occur were in the same direction as those observed in the case previously described and may be considered as confirmatory evidence. In this patient, at the start of the drug administration, there was a slight upset in water balance, but any effects of this change appeared to be completely gone by the time the drug reaction occurred. Retention of sodium and chloride was fairly definite, while retention of potassium was less definite. Speaking very conservatively, the results in this and in the other three unpublished cases support the results of Case 4.

Figure 3 is a mass plot of all 12-hour water balances and the corresponding gain or loss in body weight. It is printed because it demonstrates the fact that under the conditions of the experiments, any gain or loss of weight is composed almost entirely of water, and that there is no complicating factor of food storage to be considered in interpreting results. It is presented also to suggest to other investigators in similar



TABLE II

*Acid-base composition of the urine in Case 5*

Each day is divided into Period x (6 a.m. to 6 p.m.) and Period y (6 p.m. to 6 a.m.). All figures are for 12 hour periods. Rectal temperatures are given for noon in Period x, and midnight in Period y.

Date	Nirvanol	Rectal temperature	Water balance	Urine									Blood Chemistry			
				Total output	pH	Titratable acidity	Ammonia	Inorganic base	Chlorides	Inorganic sulphates	Phosphates	Organic acids	pH	Bicarbonate	Chlorides	Serum proteins (refractometric)
<i>July 1933</i>	<i>grams</i>	<i>°C.</i>	<i>grams</i>	<i>grams</i>		<i>m. Eq.</i>	<i>m. Eq.</i>	<i>m. Eq.</i>	<i>m. Eq.</i>	<i>m. Eq.</i>	<i>m. Eq.</i>	<i>m. Eq.</i>		<i>m. Eq.</i>	<i>per liter</i>	<i>per cent</i>
5x	0	37.3	-359	1231	5.8	13.8	17.2	71.0	46.1	19.6	25.4	18				
5y	0	36.4	+376	575	5.3	13.7	17.8	29.7	11.4	18.0	22.9	17				
6x	0	37.4	-291	1149	5.5	15.2	17.7	63.6	38.4	19.5	22.9	9				
6y	0	36.4	+331	533	5.2	13.4	17.5	26.7	11.4	18.0	21.5	15				
7x	0	37.3	-305	1160	5.7	16.3	17.6	68.0	41.5	19.7	30.2	23				
7y	0	36.6	+371	511	5.3	13.8	17.2	24.8	10.5	18.1	20.9	12				
8x	0.3	37.6	-310	1061	6.0	12.7	16.3	61.6	35.6	19.4	22.1	6	7.37	27.5	99.4	7.74
8y	0.3	36.5	+458	447	5.1	14.3	17.8	18.0	9.9	18.1	20.4	10				
9x	0.3	37.1	-254	1182	6.0	12.6	16.4	70.8	47.8	19.5	27.1	19				
9y	0.3	36.6	+402	603	5.3	12.7	16.1	29.5	11.7	16.9	19.8	17				
10x	0.3	37.3	-168	1146	5.7	14.5	16.9	59.5	31.9	18.7	24.9	16				
10y	0.3	36.6	+322	689	5.3	11.3	14.3	29.5	12.7	15.8	17.6	12				
11x	0.3	37.2	-336	1257	5.6	14.9	16.9	63.8	40.0	19.2	26.2	24	7.37	23.2	94.8	7.32
11y	0.3	36.3	+192	859	5.6	11.5	16.3	35.4	16.5	16.9	20.5	14				
12x	0.3	37.3	-164	1198	6.1	10.4	15.0	70.8	43.4	17.9	22.3	21				
12y	0.3	36.5	+196	730	5.3	11.2	16.8	29.5	14.9	16.7	17.4	14				
13x	0.3	37.6	- 92	1076	5.5	14.4	16.6	59.2	40.4	18.2	20.5	20	7.31	22.5	101.8	7.11
13y	0.3	37.0	+ 79	726	5.4	10.2	14.9	35.3	16.3	16.8	16.3	12				
14x	0.3	38.8	-192	1150	5.6	11.4	15.0	57.4	75.1	18.5	17.6	26				
14y	0	39.3	+175	785	4.9	17.9	17.0	38.4	19.8	20.2	22.4	23				
15x	0	39.9	-206	1076	5.2	17.7	20.5	52.4	29.2	20.1	23.6	32	7.36	19.2	94.4	7.71
15y R	0	39.3	+116*	332*	5.0*	12.9*	17.2*	12.8*	9.8*	16.1*	18.8*	5*				
16x R	0	39.5	- 14*	326*	5.2*	10.7*	13.1*	11.5*	6.0*	13.0*	16.4*	12*	7.33	19.2	91.4	7.78
16y R	0	37.8	+ 38*	265*	4.9*	14.7*	15.5*	10.1*	0.4*	13.6*	20.7*	14*				
17x R	0	38.0	-308	1335	5.2	18.0	29.9	26.1	7.0	19.1	25.4	10				
17y R	0	38.3	+133*	261*	5.1*	9.7*	14.9*	6.3*	2.0*	14.1*	13.1*	5*				
18x R	0	38.2	- 64*	987*	5.6*	9.1*	21.0*	28.8*	13.2*	15.9*	12.5*	13*	7.37	23.0	97.8	7.40
18y R	0	38.0	+104*	425*	5.4*	6.0*	13.5*	7.4*	4.8*	11.5*	5.3*	6*				
19x R	0	37.4	- 1	1032	5.7	8.6	24.4	32.1	15.9	17.0	16.2	21				
19y	0	36.2	- 50*	433*	5.7*	7.3*	13.8*	15.0*	9.8*	16.3*	11.3*	14*				
20x	0	36.8	- 88	884	6.1	7.4	23.4	42.1	26.0	17.6	15.4	27	7.31	25.0	97.8	7.35
20y	0	36.5	+175*	421*	5.3*	9.7*	17.9*	11.2*	6.0*	15.6*	12.5*	12*				

R after the date denotes the presence of a skin eruption.

\* denotes period of decreased intake of diet.

control period, the period of the drug reaction, or the period of partial starvation, can the pH be said to vary significantly from the normal, except, perhaps, for a slight tendency to become more acid during the period of diminished intake of the diet. In Periods 14y and 15x, there was a small reduction in the pH, hardly enough to be significant. Because of the original low level of the pH of the urine, any shift of the reaction toward the acid side would be limited.

The titratable acidity and ammonia were quite constant until Periods 14y and 15x, during which both were slightly increased. These periods were at the beginning of the fever. During the following periods, when the intake was lowered, there was a corresponding lowering of the excretion of

all the elements except ammonia, which was excreted in an amount greater than normal. There was a very marked diminution in the excretion of inorganic base and chlorides during the fever. At least part of this was due to diminished intake, but the output of these electrolytes was decreased much more proportionately than would be expected from the change in the output of phosphates and sulfates.

The output of chlorides in this case, as in Case 4, increased suddenly just at the beginning of the fever in Period 14x. This fact was remarkable because there was no increase in the excretion of cations to accompany the increased anions in the urine, nor was there any increase in the acidity. All determinations were repeatedly checked to

rule out the possibility of analytical error. No explanation can be offered at present for this phenomenon. The possibility that the chloride is excreted in combination with some strong organic base suggests itself, but has not been verified.

During the period of decreased intake of food, the ammonia output increased noticeably, apparently to take the place of fixed base. Organic acids could not be determined very accurately and appeared very irregular. The maximum excretion of 32 m. Eq. reached on Period 15x, at the beginning of the fever, while the intake was still constant, may indicate an increased oxidation of fat.

The following interpretation may be placed upon these data. The diet was fairly rich in fat and the pH of the urine tended normally to be low. It is well known that children develop signs of ketosis and acidosis much more readily than adults. It is also well known that during fever metabolism is increased. In nirvanol fever, the fuel for the increased metabolism is not protein and hence must consist of fat and carbohydrate, and probably in this case consists mostly of fat. The tendency to alkalosis, which Koehler (10) observed during fever in adults, is evidently counteracted by the above mentioned tendencies in children. The results obtained in this case are a combination of the shifts of water and electrolytes which occur during uncomplicated nirvanol fever, plus the effects of partial starvation. The important point is that no sign of any alkalosis can be observed. The results of this work may be taken to indicate that mineral metabolism during allergic states is a matter of hydration and dehydration, not of fundamental changes in the acid-base economy of the body, and that the benefits of acid therapy in allergy may be due to the diuresis and dehydration resulting therefrom.

A few other chemical analyses of the blood have been included in Table II. Bicarbonate was determined gasometrically, chlorides volumetrically, and pH by means of a special quinhydrone electrode mounted inside a glass syringe. Determinations were made on blood serum, collected under paraffin oil, and protected from contact with the air. Serum protein concentration, determined by a dipping refractometer, showed no significant trend in this particular case. Since this measurement is considerably influenced by changes in se-

rum lipids and other factors, not much emphasis should be placed upon it. The pH remained constant within the limits of experimental error both in the normal period and during the rash and fever. Bicarbonates were significantly lowered during the fever. This was probably, but not necessarily due to their displacement by some other anion. The high value for serum chlorides in Period 13x was interesting in view of the outpouring of chlorides which occurred on the following day. The lowest value for serum chlorides occurred during the height of the fever, but the significance of this was rendered somewhat less clear because of the lowered food intake at this point. These few figures add some confirmation to the finding that there is no trend toward alkalosis during the condition known as nirvanol sickness.

### *Energy metabolism*

During the study of the exchange of water it was found that the heat lost by vaporization of water remained normal or slightly below during the fever but rose markedly in the two or three days immediately following the cessation of the fever (see Figure 1). It is generally accepted that febrile temperatures are reduced through the mechanism of increased perspiration. In these children with nirvanol fever, insensible loss of water did not increase until after the fever had abated. Therefore, the excess heat produced must have been lost by increased radiation, convection, and conduction. Normally about 75 per cent of the heat produced by the body is lost by these means, and 25 per cent by evaporation of water. For the sake of completeness, studies were made of the energy exchanges of five other patients with nirvanol sickness who were not maintained in the metabolism ward.

Daily measurements were made of basal oxygen consumed and carbon dioxide produced, and simultaneous measurements were made of the rectal and skin temperatures. Skin temperature was measured over the chest and epigastrium by the method devised by Burton (11). This involves the use of a resistance thermometer made by sewing fine nickel wire to the inside of a gauze vest. In still air, the heat lost by a warm body by radiation, convection, and conduction is directly pro-

portional to the difference between the surface temperature of the body and the temperature of the environment. Changes in relative humidity over a range of 20 to 80 per cent have very little effect on this property of warm objects. Hence by measuring the average skin temperature of a patient and subtracting the room temperature, one can obtain a comparative measure of the heat which is lost by radiation, convection, and conduction. Burton (12) has shown that a measure of the ease with which heat is conducted from the interior of a warm body to the outside can be obtained by calculating the ratio

$$\frac{\text{Excess of skin temperature over room temperature}}{\text{Excess of rectal temperature over skin temperature}}$$

Changes in the ease with which heat is conducted from the interior to the exterior are chiefly due to changes in the peripheral blood flow, and accordingly this index is a comparative measure of changes in the circulation of the skin. This index will be referred to as the circulation index, and the numerator of the fraction as the excess temperature.

TABLE III

Subject J. S.

Weight 29.2 kgm. Ht. 131 cm. Treated with nirvanol but developed no reaction.

Date	Room temperature	Skin temperature	Excess temperature	Rectal temperature	Circulation index	Metabolism
March 1933	°C.	°C.	°C.	°C.		cal. per sq. m. per hour
5	22.20	33.40	11.20	37.5	2.7	43.8
6	23.15	32.15	9.00	37.0	1.9	45.1
7	27.46	33.96	6.50	37.4	1.9	45.6
8	24.52	33.11	8.59	37.2	2.1	48.4
9	19.38	31.25	11.87	37.5	1.9	42.4

Table III shows first the results of an experiment which will serve as a control. Boy J. S. received nirvanol but failed to develop any reaction. The room temperature was deliberately varied from day to day. From the table it is apparent that the excess temperature varies with the room temperature, but the circulation index remains relatively constant. These figures show that over the time and temperature range of the experiment, there was no significant alteration by the normal body of the peripheral circulation.

TABLE IV

Subject W. L.

Weight 23.6 kgm. Ht. 128 cm. Energy metabolism during nirvanol sickness and a subsequent respiratory infection.

Date	Room temperature	Skin temperature	Excess temperature	Rectal temperature	Circulation index	Metabolism	Remarks
December 1932	°C.	°C.	°C.	°C.		Cal. per sq. m. per hour	
21	25.5	33.5	8.0	36.7	2.5		
22	27.5	35.1	7.6	36.7	4.7	42.7	
24	28.1	36.0	7.9	37.6	4.9	44.7	
25	27.2	35.9	8.7	38.2	3.7	51.9	
26	26.4	37.5	11.1	39.0	7.4	54.0	Rash
27	27.2	37.7	10.5	38.4	15.0	45.2	Rash
28	27.5	36.9	9.4	38.6	5.5	54.1	Rash
29	26.6	34.6	8.1	38.2	2.3	53.1	Rash gone
30	27.0	35.9	8.9	38.7	3.2	45.5	
31	26.7	34.3	7.6	37.2	2.6	44.7	

In contrast, Boy W. L. developed a typical reaction lasting three days from December 25, 1932 to December 27, 1932. The second rise in temperature on December 28, 1932 was due to the development of a respiratory infection. From an experimental standpoint this was a fortunate occurrence since it permitted a comparison of the effects of fever, with and without a rash, on the circulation index. In this experiment the room temperature was kept relatively constant. During the nirvanol rash and fever the excess temperature and the circulation index were both significantly increased, while later, during the fever due to the respiratory infection and after the disappearance of the rash, the excess temperature and index returned to normal. This case showed the most pronounced changes; others studied showed changes of lesser magnitude, but in the same direction. From the data observed the conclusion might be drawn that during the abatement of nirvanol fever the excess heat is lost, not by the usual method of increased perspiration, but by increased peripheral circulation, and hence increased loss through radiation, convection, and conduction. The rash and visible flushing of the skin may be considered as the mechanism whereby the increased heat loss is brought about.

The measurements of heat production during the fever have been calculated as per cent of the normal level established on each child during the

afebrile state. Comparison of all the results shows that there is an increase of approximately 13 per cent in the heat produced for each rise of one degree centigrade in the rectal temperature. The exchange of energy during malaria, typhoid, erysipelas, and other fevers has been worked out in great detail by Barr, DuBois, and their co-workers (13) at the Russell Sage Institute. They found in general that the heat production during fever remained at about 20 to 28 per cent above basal except during chills, when it rose to 200 per cent above basal. Fever was maintained because of inadequate increase of heat loss through the skin, not because more heat was produced than can normally be eliminated. This mechanism is apparently common to all fevers, including nirvanol fever.

It may be said therefore that nirvanol fever is due mainly to inadequate increase of heat loss, and is relieved chiefly by a later increase in heat radiated, conveyed, and conducted away from the body. This last process is doubtless brought about by the changes in the peripheral circulation which are evidenced by the erythema during the skin reaction. We infer that in the early stages evaporation is prevented from increasing, and that this is the chief reason for the fever.

#### SUMMARY

1. There occurred in phenylethylhydantoin sickness, as in serum sickness, a temporary retention of water, then of sodium and chloride, then of potassium, in the order stated.

2. During the fever, there was a slight tendency toward acidosis, as evidenced by a study of the acid-base equilibrium of the urine. At no time during the reaction did there appear any sign of alkalosis.

3. The rate of oxygen consumption was increased in proportion to the height of the fever.

4. The fuel for the increased heat production was not protein.

5. The fever was relieved by an increase in the

heat lost through the channels of radiation, conduction, and convection.

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# OBSERVATIONS UPON THE CALCIUM AND PHOSPHORUS METABOLISM IN A CASE OF ACROMEGALY SHOWING MARKED OSTEOPOROSIS

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Our interest in the mineral metabolism in acromegaly was aroused by the remarkable generalised rarefaction of the bones shown in x-ray films of one of our patients in an advanced state of this disease. Since we have been unable to find in the literature any record of metabolic studies in acromegalics more recent than that of Bergeim, Stewart and Hawk (1) in 1914, and in view of the important advances of the past few years in the study of calcium and phosphorus metabolism, particularly in certain pathological conditions of bone, we feel that the observations which we have been able to make on this patient are worthy of recording.

## CASE HISTORY

T. W., a Jewess 51 years of age, was admitted to the metabolism service of the Royal Victoria Hospital on

April 6th, 1932, complaining of weakness and failing vision. Her appearance was that of an advanced acromegalic (Figure 1).

According to the history obtained from the patient herself, she was born in Russia, and in her early life was a strong, healthy, apparently normal girl. At the age of eleven she began to be troubled with severe generalized headaches which ceased coincidentally with the onset of menstruation two years later. She was married at 20 and had two normal children, in her 22d and 24th year respectively.

At the age of 29 she came to Canada; she remembers that at this time she was admired for her general physique and attractive appearance. Until she reached the age of 32 her menstruation had always been normal, but at this time it ceased completely and finally. In the following year, several years after the death of her first husband, she married again, but had no further pregnancies. Shortly after this, her sister, who had not seen her for four years, failed to recognize her, as her fea-



FIG. 1.



FIG. 2.

FIGS. 1 AND 2. APPEARANCE OF PATIENT IN 1932.



FIG. 3. X-RAY OF RIGHT HAND.

tures had enlarged, and changed in appearance, as also had her hands. A photograph taken in her 26th year shows evidences of early acromegaly; the characteristic appearance is well established in one taken four years later. She states that her face has been changing progressively since the onset. At the age of 39 she was forced to wear a larger size of shoes, but there has been no change in size since then.

In her late forties a generalized feeling of weakness began which has been progressive since, especially in the legs; she now has to walk with a cane. When she was about 48 her eyesight began to fail, and for the two years preceding admission to hospital she has been unable to read.

Her hair, which used to be oily, has now become dry and coarse; the skin is less greasy than it was, but is not dry.

In the physical examination the most striking feature is the advanced acromegalic appearance; the features are large and coarse, with heavy overhanging supraorbital ridges, broad cheek bones, large nose, thick lips, and enormous projecting mandible. The hands and feet are also much enlarged, and typically spade shaped. The chest is definitely increased in anteroposterior diameter, the supra- and infra-clavicular fossae being very pronounced. The pelvic organs are also large; neither external nor internal genitalia show the changes usually associated with the menopause. The pelvic hair has the normal female type of distribution, and is moderately coarse; that of the head is decidedly coarse, dry and very abundant; the axillae, however, have but scanty hair.

While the optic fundi appear normal, vision in the left eye is 6/30, in the right 6/20; the visual fields show a bitemporal hemianopsia. Basal metabolic rate, averaged from several determinations, is  $+3$  (approximately 1500

calories per 24 hours). Blood chemistry is normal for the main constituents. The urine is negative. There is a moderate degree of anaemia, with 3,400,000 erythrocytes, and 60 per cent haemoglobin (8.4 grams per 100 cc.). The blood pressure averages 130 systolic and 85 diastolic. There is nothing of importance in the other organs.

Numerous x-ray plates were made all of which showed a spongy osteoporotic appearance of the bones. The following is a somewhat abbreviated summary of the reports furnished by Dr. A. H. Pirie, Roentgenologist-in-Chief of the Royal Victoria Hospital.

*Hand*—The terminal phalanges have a corn sheaf appearance. The base of each middle phalanx is expanded and lipped, externally and internally. The trabeculae of the phalanges, metacarpal and carpal bones are opened out so as to form a wide network. There is a periosteal new growth of bone on the shaft of each proximal phalanx and metacarpal bone.

*Foot*—The trabeculae are opened up in all bones of the foot except the shafts of the metatarsals and the proximal phalanges. There is periosteal new bone formation in the shaft of the first left metatarsal bone.

*Skull*—Stereoscopic views show a great thickening of the calvaria, especially in the regions of the parietal bones. The thickening of the skull is like a dense spongework, and not solid ivory bone. The frontal sinuses and antra are especially large. The sella turcica is enlarged and presents an eroded appearance. Side views of the head show a great increase in the size of the bones of the face. The cervical vertebrae present a rarefied ground-glass appearance.

*Lower extremities*—The bones are rarefied and irregular in outline, the trabeculae are opened up and form a sponge-like network, the spaces of which are larger than

in normal bone. The lower ends of the femora at the metaphyses are flattened from before backward and broadened from side to side. The articular surface at the lower end of each femur is more irregular in outline than normal; such an appearance is observed in cases of osteoarthritis, but evidence of lipping, as in osteoarthritis, is absent. There is marked periosteal new bone formation in each femur, broadening the diameter of the shaft. The patellae are a little larger than usual.

*Chest*—The heart is under average size; the aorta, trachea, and diaphragm are negative. The trabeculae of the bones of the chest and clavicles present the opened-out appearance of a meshwork.

#### GENERAL METHODS

The metabolic studies reported here were carried out during two different periods in hospital, at an interval of approximately one year, during which time the patient had been under observation in the Out-Patient Clinic, where no obvious changes in physical condition were noted. In May, 1932, Diet I was commenced, and the observations were carried out over three periods of three days each. Following this, Diet II was given and after a fore period of several days, observations were made for the same number of periods. The patient was readmitted in May, 1933, as we desired to see the effect of a diet still higher in calcium, with and without viosterol; accordingly observations were made over three periods during which she consumed Diet III, followed by two periods in which in addition she was given viosterol (250 D) in the dose of twenty drops three times daily, and three periods with thirty drops at each dose.

The composition of the individual diets was as shown in Table I; it did not vary during the period under study. According to standard tables (2), Diets II and III were approximately neutral; Diet I, however, was found to have an excess acidity of approximately 18 cc. of normal acid, to counteract which sodium bicarbonate was given to the patient immediately after meals in dosage calculated to neutralize the excess acidity. At no time was the nitrogen balance on the negative side.

The nursing, dietetic and analytical methods used were the same as those described in a previous communication (3).

#### RESULTS

The results of this investigation are summarized in Table II, in which the first two sets

TABLE I

*Composition of diets*  
(Daily intake in grams or cubic centimeters)

	Diet I	Diet II	Diet III
Orange.....		250	250
Grapefruit.....	100		
Oatmeal (cooked).....		150	150
Cream of wheat (cooked).....	150		
Potato—white.....	50	50	50
Potato—sweet.....	100	100	100
Carrots.....		100	100
Bread.....	140	120	120
Butter.....	40	24	24
Eggs.....	2*	2*	2*
Egg white.....	81	0	0
Milk.....	100	475	675
Honey.....	18	17	17
Canned peach.....		155	155
Tomato juice.....	150		
Apple.....	100		
Sugar.....	32		
Soda cracker.....			10
Approximate values—Protein.....	50	50	57
Fat.....	50	50	59
Carbohydrate.....	200	200	217
Calcium content.....	0.299	0.810	1.060
Phosphorus content.....	0.533	0.966	1.259

\* Eggs were not weighed; those used were all approximately the same size.

of data represent the observations made in 1932 and the remaining sets of data those made in 1933. It will be observed that the blood serum calcium and inorganic phosphorus remained fairly constant throughout all periods of investigation, the blood calcium values tending to range from normal to slightly below normal, while the inorganic phosphorus was slightly elevated.

From the standpoint of the balance of both calcium and phosphorus the important point is that, disregarding the periods in which viosterol was given, the balance was decidedly negative on the diet affording the lowest intake of calcium and phosphorus, less negative on Diet II, while it became positive on the higher intake of Diet III. In Table III are summarized the reports of calcium and phosphorus balances in acromegaly which we have been able to find in the literature from the work of Schiff (4) in 1897, to the studies of Bergeim, Stewart and Hawk in 1914 (1). Our own figures are found in the first three sets of data. To make the results comparable, all values have been recalculated in terms of grams of calcium or phosphorus per diem. In only one

TABLE II

Calcium and phosphorus metabolism. (In the case of diet, urine, and feces, figures represent the average for a single period of three days expressed in milligrams)

Diet	Number of periods	Intake	Output			Balance	Serum
			Urine	Stool	Total		
Calcium							
		<i>mgm. per 3 days</i>	<i>mgm. per 3 days</i>	<i>mgm. per 3 days</i>	<i>mgm. per 3 days</i>	<i>mgm. per 3 days</i>	<i>mgm. per 100 cc.</i>
I.....	3	897	968	988	1956	- 1059	9.2
II.....	3	2430	1004	1628	2632	- 202	11.0
III.....	3	3180	1243	1664	2907	+ 273	10.2
III + Viosterol.....	2	3180	1370	1637	3007	+ 173	10.0
III + Viosterol.....	2	3180	2416	1151	3567	- 387	10.6
Phosphorus							
I.....	3	1599	1390	809	2199	- 600	5.1
II.....	3	2898	1790	1366	3156	- 258	4.1
III.....	3	3777	2296	1276	3572	+ 205	4.1
III + Viosterol.....	3	3777	2068	1336	3404	+ 372	4.3
III + Viosterol.....	2	3777	3280	1138	4418	- 641	5.1
							5.0

case reported completely enough to be included in the table was a negative balance found—that of Oberndörffer (7) reported in 1908, in which, with a calcium intake of 1.354 gram per diem the sub-

TABLE III

Summary of calcium and phosphorus metabolism data found in the literature. (Values represent the average for a single day expressed in milligrams of Ca and P.)

Author	Diet	Intake	Output			Balance
			Urine	Stool	Total	
Calcium						
Seiver and Bryan. ....	I	mgm. 299	mgm. 323	mgm. 329	mgm. 652	- 353
	II	810	335	543	878	68
Von Moraczewski (5) .....	III	1090	414	555	969	+ 91
	I	1606	329	526	855	+ 751
Edsall and Miller (6) .....	II	2170	178	701	879	+1276
Oberndörffer (7) .....		1691	632	572	1334	+ 137
Medigreceanu and Kristeller (8) ..		1354	409	1571	1980	- 626
Bergeim, Stewart and Hawk (1) ..		1100	626	242	868	+ 232
		1067	114	781	895	+ 172
Phosphorus						
Seiver and Bryan. ....	I	533	463	270	733	- 200
	II	966	397	456	1053	- 87
Schiff (4) .....	III	1259	765	425	1190	+ 69
Von Moraczewski (5) .....	I	1120	742	255	1027	+ 93
	II	3478	1704	244	1948	+1530
Edsall and Miller (6) .....		4269	2022	258	2310	+1959
Oberndörffer (7) .....		1355	762	334	1096	+ 259
Medigreceanu and Kristeller (8) ..		1001	628	356	984	+ 17
Bergeim, Stewart and Hawk (1) ..		675	556	79	665	+ 10
		750	400	235	635	+ 115

ject actually excreted 0.626 gram daily more than he ingested. In this same case the phosphorus balance was just barely positive (a retention of 0.017 gram per day). It will be observed on studying the figures that no case in the literature received a daily calcium intake as low as did our patient with Diets I and II; and that with Diet III, when her daily calcium intake was comparable to that fed by Medigreceanu and Kristeller (8) and by Bergeim, Stewart and Hawk (1), her daily retention was 0.091 gram as compared with their respective values of 0.232 gram and 0.172 gram. A study of the phosphorus intake summarized in Table II leads to similar considerations except that the case reported by Medigreceanu and Kristeller (8) on an intake of 0.675 gram of phosphorus a day, slightly higher than that afforded by our Diet I, remained in positive balance with a retention of 0.01 gram a day. In all studies except the present one, the intake of calcium and phosphorus has been so high that a positive balance might be expected in a normal individual (3).

After our subject had received Diet III for three periods of three days each, she was given viosterol, in addition, for five periods to observe its effect on the absorption of calcium and phosphorus. For the first two viosterol periods the



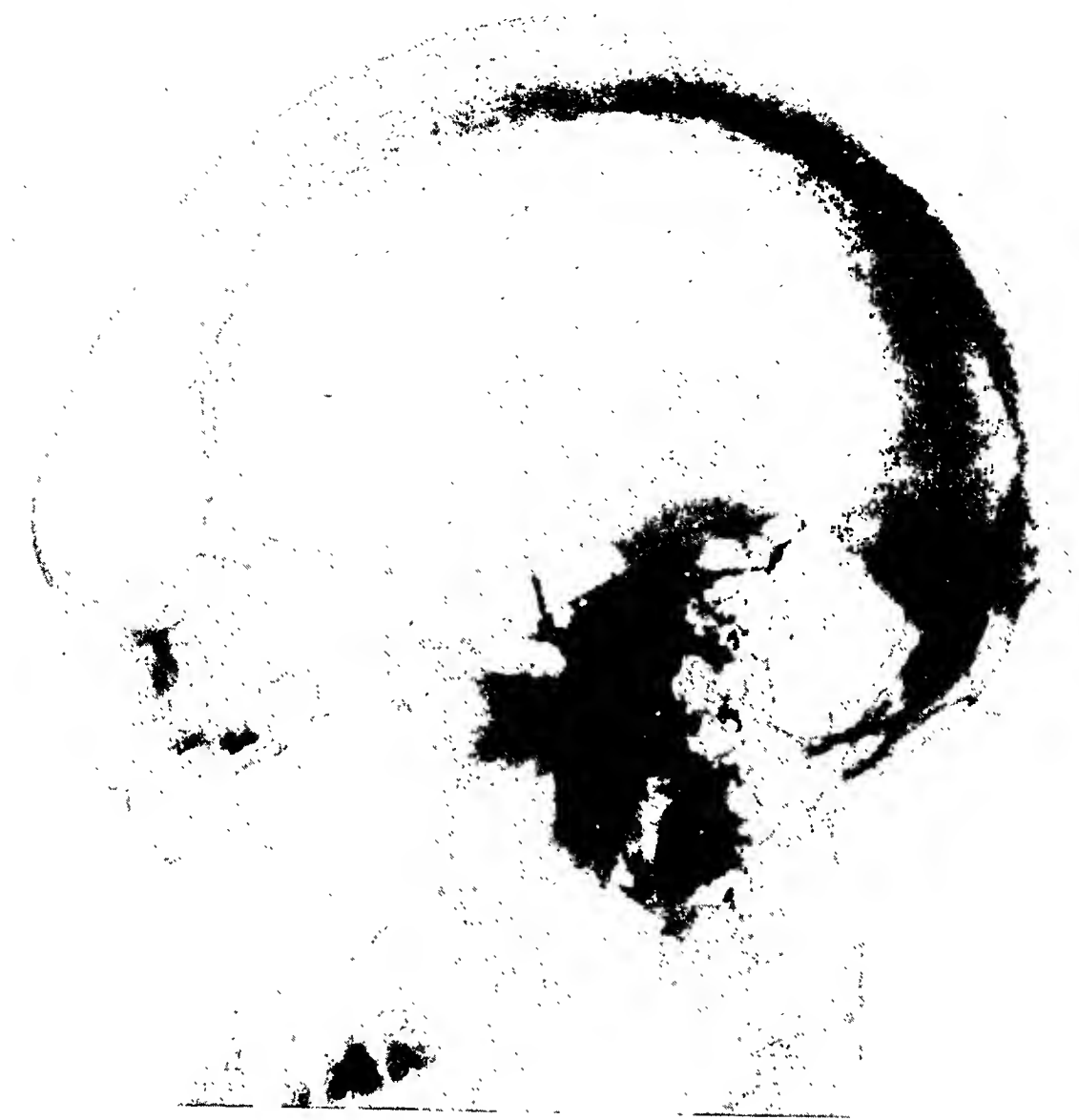


FIG. 4. X-RAY OF SKULL.

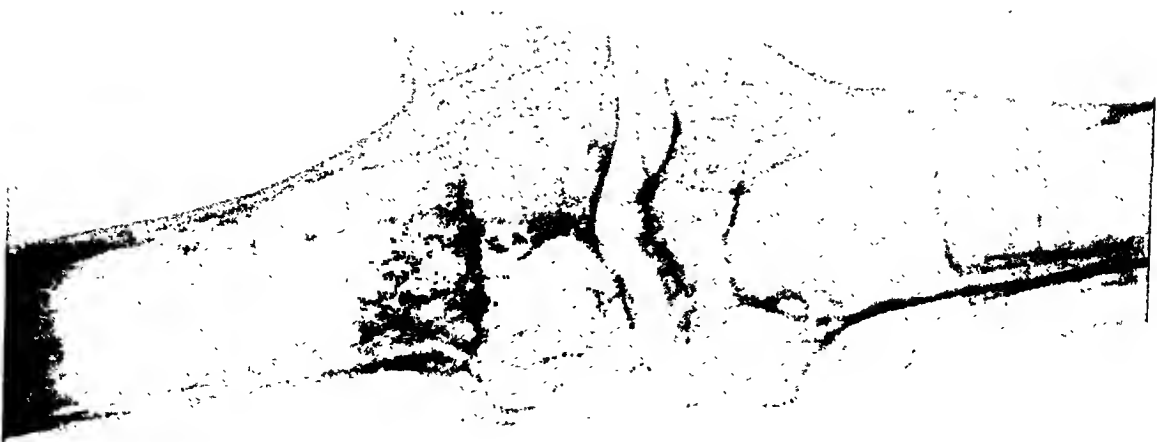


FIG. 5. X-RAY OF LEFT KNEE REGION.

dosage was sixty drops of the 250 D preparation a day (twenty drops three times a day), and for the last three periods she received ninety drops a day divided into three equal doses. A study of the data shows that during the last two of the five viosterol periods the excretion of both calcium and phosphorus in the urine increased significantly, whereas there was a decrease in the amount in the faeces; the increased urinary excretion, however, was so great as to swing the balance to the negative side. Except for the increase of phosphorus in the urine, these results are similar to those obtained by Pugsley (9) in rats receiving large doses of irradiated ergosterol, relatively many times greater than those received by our patient. We are unable to offer an explanation for this effect.

Under this therapy the level of both elements in the serum tended to rise slightly, but did not exceed those observed in the preceding periods without medication.

#### DISCUSSION

In their monograph on the pathological findings in acromegaly, Cushing and Davidoff (10) emphasize the adenomatous changes found in the endocrine organs. In two of their cases, where the parathyroids were identified, in one a single parathyroid was found enlarged and showed microscopically evidence of proliferative activity; in the other, what was taken to be a central adenoma of the parathyroid was found. They were, however, unable to find in the literature any extensive reference to the state of the parathyroids in acromegaly, and point out that our knowledge of the function of the parathyroids is greater than our knowledge of their histopathology.

Our present studies fail to produce definite metabolic evidence of parathyroid hyperfunction, the criteria of which are now so well established (11) (12). The failure to demonstrate an increase of the serum calcium above 11 mgm. per 100 cc. or a decrease of the inorganic phosphorus below 4.1 mgm. per 100 cc. is evidence against the presence of this condition, even in its less typical forms (13). On the other hand it is obvious that the excretion of calcium in the urine is relatively greater in amount than that observed in normal individuals on similar intakes of calcium, and is one of the factors which tend to produce a

negative balance when the intake is low. As the diets were approximately neutral this increased excretion cannot be due to "uncovered" acid radicals.

Unfortunately in none of the cases here reviewed are figures given for calcium and phosphorus in the serum. The cases of Edsall and Miller (6) and Medigreceanu and Kristeller (8) show an excretion of calcium in the urine which approaches the levels seen in frank hyperparathyroidism; those of Von Moraczewski (5) and Oberndörffer (7) are nearer to those found in our case. Von Moraczewski's second observation and Bergeim, Stewart and Hawk's (1) case show levels similar to those found in normal subjects.

Whether a mere increase of excretion of calcium in the urine, without changes in the calcium and phosphorus levels in the serum, can be taken as evidence of hyperparathyroid activity or not is at present a controversial subject, with the bulk of the evidence favouring the negative. With the present criteria, our case, lacking the abnormal findings in the serum, and showing only a moderate increase in urinary excretion, cannot be classified as showing true evidence of parathyroid hyperfunction.

The calcium metabolism is similar in our case to that observed in Cushing's (14) twelfth case of pituitary basophilism in that both show a normal level in the serum, and a moderately increased excretion in the urine. The serum phosphorus, however, in Cushing's case was at a definitely low level, whereas it was within normal limits in ours.

It is a well recognized fact that rarefaction of the bones may result from hyperthyroidism (15), a condition which might be considered as an etiological factor in this case. There is, however, nothing in the history or physical condition to suggest past or present hyperthyroidism, the patient has never been under thyroid medication, and the basal metabolic rate falls within normal limits. From the metabolic point of view the excretion of calcium and phosphorus is also at a much lower level than that found in hyperthyroidism.

The occurrence in acromegaly of such marked rarefaction of the bones as is present in our case must be unusual, as we have been unable to find any reference to it in the literature. Studies of the histopathological changes found in the bones

of acromegalics (10, 16, 17, 18, 19) indicate that the skeletal growth is due chiefly to new compact bone laid down by the periosteum, with coincidental resorption of the old bone on the inner side, leading to enlargement of the marrow cavities in the long bones, accessory sinuses in the face, and general broadening of the flat bones. In the thorax, growth takes place by the formation of new bone at the end of the ribs through proliferation and subsequent ossification of the costal cartilages. Most of these changes are evident in x-ray studies of our case. Thus, although the epiphyses are closed, the skeleton of the acromegalic grows in breadth, the calcium and phosphorus being laid down in the newly ossified tissue and resorbed from the old bone, in a process which appears from the biochemical viewpoint to be similar to that involved in the growth of bone, in normally growing young animals.

It is well established that the young growing animal, or one in the latter part of pregnancy, is sensitive to alterations in the calcium or phosphorus or vitamin intake, and a deficiency in these elements due to a poorly balanced diet or to poor absorption may lead to rickets or osteomalacia with generalized rarefaction of the bones.

In our case Diet I is deficient in calcium for the average adult, Diet II probably so, and Diet III theoretically sufficient. The increased excretion of calcium and phosphorus in the urine together with the change from negative to positive balance with increase in intake would tend to show that in this case absorption is not at fault. The failure of viosterol therapy to increase the retention of calcium may be taken as evidence that the lack of vitamin D is not a significant factor, at least with these diets. Our studies show that in her present condition, when the overgrowth of bone is not rapid, though x-ray suggests that it is still proceeding, the patient needs a daily intake of approximately one gram of calcium to remain in positive balance; with less than this there is actual calcium loss. While it is obviously impossible to obtain a reliable dietary history extending over the past twenty years, we feel that the most rational explanation of the bony rarefaction is that during the prolonged period of overgrowth of the skeleton stimulated by pituitary dysfunction, the calcium-phosphorus intake has been insufficient, when associated with the increased urinary excre-

tion of these elements, to establish a positive balance of sufficient degree to meet the demands of the growing bones. In consequence these have been laid down in a state of incomplete calcification, a state which has not since been remedied by any change in the diet sufficient to produce a positive balance, and promote storage of calcium triphosphate in the bones.

#### SUMMARY

Calcium and phosphorus metabolism has been studied in an advanced case of acromegaly showing generalised rarefaction of the bones. These findings are compared with others from the literature, in which, however, no other reports of rarefaction of the bones have been found.

It is suggested that in this case the osteoporosis is due to an insufficient intake of calcium and phosphorus associated with a moderate increase in excretion in the urine during the years of acromegalic growth; no complete evidence has been found to support the view that parathyroid or thyroid hyperfunction plays any part in the process.

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# A NOTE ON THE METABOLIC CRITERIA OF HYPERPARATHYROIDISM

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As might be anticipated the identification of parathyroid hyperfunction as the cause of osteitis fibrosa cystica (21, 30) has occasioned many attempts to indict the parathyroid glands in other malacic diseases of the bone. The controversy in the literature regarding the use of parathyroidectomy in the treatment of various disorders of the skeletal system would seem to be ample testimony that the possible rôle of the parathyroids in causing diseases associated with disturbances of calcification is not yet clearly defined (3, 4, 5, 6, 7, 15). In the opinion of some writers apparently, backache and some x-ray evidence of demineralization of the vertebrae are sufficient grounds for the diagnosis of "parathyroidism" and the procedure of parathyroidectomy. The more critical school has maintained that hyperparathyroidism is a distinct clinical entity usually associated with adenoma of parathyroid tissue and characterized by a definite disturbance of calcium and phosphorus metabolism. Certain metabolic criteria have been proposed which are:

1. Hypercalcemia, usually with hypophosphatemia.
2. Excessive excretion of calcium and phosphorus.
3. Increased retention of calcium with high phosphorus intake.

Frequently it is implied that some one or all of these criteria must be satisfied before a diagnosis of hyperparathyroidism may be made; occasionally the statement is made that the laboratory findings are "pathognomonic" of the disease (15).

The present communication does not pretend to settle the controversy regarding etiologic relationships; original data are submitted simply to emphasize the hazards of entertaining any notions that the biochemical findings in diseases of the skeleton can suffice to establish a diagnosis.

## METHODS

Protocols of the cases studied are appended. Cases 1, 2 and 3 were diagnosed myelomatosis; in Case 1 the diagnosis was confirmed by autopsy, in Cases 2 and 3 by biopsy of excised bone. Case 4 presented the clinical picture of Paget's disease of the bones and this diagnosis was confirmed at autopsy.

The patients, all males, were studied on the medical wards of the New Haven Hospital. The diets were specially prepared in the diet kitchen of the hospital and the usual precautions required for careful balance studies were observed. In Case 1, since varied diets were given, the intakes of calcium and phosphorus were calculated from Sherman's tables (36). In Cases 2, 3 and 4 a constant intake was insured by giving an identical diet on every day of the experiment as well as during a three-day fore-period. At frequent intervals duplicate diets were analyzed for calcium, phosphorus and nitrogen. The actual and calculated values for calcium and phosphorus in the low calcium diets agreed within 5 mgm. for 24 hours. In the high calcium diets the analytical values were lower than the calculated figures by 2.3 per cent for calcium and 7.3 per cent for phosphorus. The acid-base values of the diets were essentially neutral.

In Cases 2 and 4 the excreta were collected in periods of three days each; in Case 3 the periods were 4 days each; a few periods in Case 1 were longer. The principles of the ward routine and methods closely followed those described by Bauer and Aub (9). In the collection, separation and preservation of specimens the usual careful techniques were employed. Daily analyses for creatinine served as checks on the completeness of urine collections.

The wet stools for each period were mixed according to a method used in this laboratory (26). Weighed aliquots of urine and stools were ashed in a muffle furnace at 600° C. Calcium was precipitated as the oxalate from acid extracts of the

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ash at a pH between 4 and 5. After standing overnight the salt was filtered off on fritted Jena-glass funnels, washed with dilute ammonia water, dissolved in sulphuric acid and the oxalate was determined by titration with 0.05 *N* potassium permanganate. Phosphorus was precipitated from the extract of the ash as magnesium ammonium phosphate. After cooling, shaking for five minutes and allowing to stand overnight, the salt was transferred quantitatively to weighed Gooch crucibles and ignited in the muffle furnace at 600° C. for one hour. After drying in the desiccator the crucibles containing magnesium pyrophosphate

were reweighed. The accuracy of the methods for calcium and phosphorus in the excreta was frequently checked by analyses of known solutions. The maximum error was 3 per cent.

Serum calcium was determined by a modification of the Kramer-Tisdall method (20) and serum phosphorus by the Fiske and Subbarow method (17). Serum proteins were determined by the previously described method in use in this laboratory (12).

## RESULTS

The detailed studies of each case are recorded in Tables I to IV. To facilitate comparison with

TABLE I  
Calcium and phosphorus balance. (Case 1, J. B. Diagnosis: Multiple myeloma)

Dates (inclusive)	Period	Balance per three-day period										Serum					
		Calcium					Phosphorus					Cal- cium	Phos- phor- us	Non- pro- tein nitro- gen	Total pro- tein	Albu- min	Glob- ulin
		Output			In- take	Balance	Output			In- take	Balance						
		Urine	Stools	Total			Urine	Stools	Total								
1930		grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	mgm. per cent	mgm. per cent	mgm. per cent	grams per cent	grams per cent	grams per cent
July 19.....	I											18.1	5.1	56	13.1	2.8	10.3
July 23.....												15.5	2.3		11.6	2.9	8.7
Aug. 5.....												11.3	3.2		11.6	2.6	9.0
Aug. 13.....												12.4	3.6	41	12.0	2.7	9.3
Aug. 19-25.....		.97	4.31	5.28	6.42	+1.14	1.95	2.76	4.71	5.13	+ .42	12.8	1.8	39	11.3	2.2	9.1
Aug. 26.....	II																
Sept. 26-29.....		.67	4.16	4.83	6.87	+2.04	1.52	1.16	2.68	4.89	+2.21						
Sept. 30-Oct. 4.....	III	.81	5.95	6.76	6.18	-.58	2.57	2.41	4.98	7.59*	+2.61						
Oct. 4-7.....	IV	1.27	6.51	7.78	6.84	-.94	2.90	2.10	5.00	8.07*	+3.07						
Oct. 10.....	V											12.6	5.5		12.2	3.1	9.1
Oct. 24-30.....		.68	3.10	3.78	5.49	+1.71	1.53	.78	2.31	3.51	+1.20	15.9	3.4	51	12.3	2.8	9.5
Oct. 31.....	VI																
Nov. 11-14.....		1.48	4.63	6.11	3.81	-2.30	1.26	1.95	3.21	1.92	-1.29						
Nov. 24-27.....		1.11	4.62	5.73	6.27	+ .54	2.44	2.01	4.45	4.74	+ .29	11.7	3.4	34	9.8	2.7	7.1
Jan. 28, 1931.....	VII																

\* 2.82 grams P per three-day period added as  $(\text{NH}_4)_2\text{HPO}_4$ .

TABLE II  
Calcium and phosphorus balance. (Case 2, A. C. Diagnosis: Multiple myeloma)

Dates (inclusive)	Pe- riod	Balance per three-day period									Serum						
		Calcium					Phosphorus				Calcium	Phosphorus	Non-protein nitrogen	Total protein	Albumin	Globulin	
		Output			In-take	Balance	Output			Intake							Balance
		Urine	Stools	Total			Urine	Stools	Total								
1933		grams	grams	grams	grams	grams	grams	grams	grams	grams	mgm. per cent	mgm. per cent	mgm. per cent	grams per cent	grams per cent	grams per cent	
Dec. 12.....	I										11.3	4.7	41	6.6	4.6	2.0	
Dec. 18.....											10.5	5.1	44	6.8	4.9	1.9	
Dec. 21-24.....		1.56	2.94	4.50	3.23	-1.27	3.80	1.36	5.16	4.16	-1.00						
Dec. 24-27.....		1.28	5.54	6.82	3.82	-3.00	3.52	2.52	6.04	4.87	-1.17						
Dec. 28.....												10.6	5.0	39	6.0	4.3	1.7
Dec. 27-30.....	II	1.18	2.87	4.05	3.83	-.22	6.16	3.78	9.94	11.64*	+1.70						
Dec. 30-Jan. 2, 1934	III	1.13	4.45	5.58	3.83	-1.75	7.13	5.61	12.74	11.64*	-1.10						
Jan. 3.....	IV										10.9	4.8	33	6.7	4.4	2.3	
Jan. 8.....											11.2	9.7	88	6.0	4.3	1.7	

\* 6.72 grams P per three-day period added as  $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ .

TABLE III  
Calcium and phosphorus balance. (Case 3, F. A. Diagnosis: Multiple myeloma)

Dates (inclusive)	Period	Balance per three-day period										Serum					
		Calcium					Phosphorus					Calcium	Phosphorus	Non-protein nitrogen	Total protein	Albumin	Globulin
		Output			In-take	Balance	Output			In-take	Balance						
		Urine	Stools	Total			Urine	Stools	Total								
1934		grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	mgm. per cent	mgm. per cent	mgm. per cent	grams per cent	grams per cent	grams per cent
Apr. 3.....	I											10.5	3.8	25	7.8	5.2	2.6
Apr. 19.....												10.3	4.5	38	7.4	4.7	2.7
Apr. 27.....												10.3	4.5	36	6.6	4.0	2.6
Apr. 27-May 1....	II	1.85	4.12	5.97	3.90	-2.07	4.21	1.91	6.12	4.98	-1.14	10.5	6.6	38	7.2	4.2	3.0
May 1.....		2.06	2.57	4.63	3.45	-1.18	4.81	1.11	5.92	4.06	-1.86	10.5	6.6	38	7.2	4.2	3.0
May 1-5.....		2.01	2.66	4.67	1.72	-2.95	3.37	1.27	4.64	2.32	-2.32	10.3	5.5	32	7.1	4.3	2.8
May 5-9.....	III											10.8	3.8	29	7.1	4.3	2.8
May 9.....												18.8	3.1	52	6.8	4.7	2.1
May 21.....												17.7	3.8		6.4	4.0	2.4
June 6.....		2.34															
June 7-10.....																	
June 8.....		1.74															
June 15-18.....		1.74															
June 18-21.....																	

TABLE IV  
Calcium and phosphorus balance. (Case 4, W. C. Diagnosis: Paget's disease)

Dates (inclusive)	Period	Balance per three-day period										Serum					
		Calcium					Phosphorus					Calcium	Phosphorus	Non-protein nitrogen	Total protein	Albumin	Globulin
		Output			In-take	Balance	Output			In-take	Balance						
		Urine	Stools	Total			Urine	Stools	Total								
1932		grams	grams	grams	grams	grams	grams	grams	grams	grams	mgm. per cent	mgm. per cent	mgm. per cent	grams per cent	grams per cent	grams per cent	
Dec. 22.....	I										10.2	4.5	38	6.7	4.5	2.2	
Dec. 31-Jan. 3, 1933		.98			.23		1.75			1.84		10.1	3.9	32	6.4	4.0	2.4
Jan. 3.....	II																
Jan. 3-6.....		.83	.97	1.80	.23	-1.57	1.51	.72	2.23	1.84	— .39	9.7	3.9	36	6.2		
Jan. 6.....	III <sup>1</sup>																
Jan. 6-9.....		.47	.96	1.43	.23	-1.20	2.67	4.17	6.84	8.42	+1.58	9.4	4.7	41	6.2	4.0	2.2
Jan. 9.....	IV <sup>1</sup>																
Jan. 9-12.....		.21	.27	.48	.26	— .22	6.04	1.04	7.08	8.64	+1.56	10.2	4.2	36	5.4	3.9	1.5
Jan. 12.....	V <sup>1</sup>																
Jan. 16-19.....		.34	.60	.94	.26	— .68	4.04	2.16	6.20	8.58	+2.38						
Jan. 19-22.....	VI <sup>1+2</sup>																
Jan. 22-25.....		.24			1.77		4.70			8.70							
Jan. 25.....	VII <sup>1+2</sup>																
Feb. 9.....		.26			1.76		5.70			8.61		10.1	3.7	28	6.1	2.7	2.0
Mar. 4.....											7.6	3.7	35	5.3	2.6	2.7	

<sup>1</sup> 16.72 grams P per three-day period added as  $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ .

<sup>2</sup> 1.50 gram Ca per three-day period added as  $\text{Ca}(\text{C}_2\text{H}_3\text{O}_2)_2 \cdot 5 \text{H}_2\text{O}$ .

During four-day interval between Periods IV and V diet and medications continued as in Period IV.

each other and with similar studies in the literature the figures for all the balance experiments have been calculated for three-day periods.

The patterns of the concentrations of calcium, phosphorus and proteins in the sera of the four patients varied widely. In Case 1 the hypercalcemia was accompanied by hyperproteinemia while the terminal elevation of serum calcium in Case 3 occurred with a slightly diminished concentration

of serum proteins. In all cases serum phosphorus and proteins fluctuated considerably, but the changes apparently bore no relation to serum calcium except in Case 4 where a progressive decrease in the serum proteins was reflected in a diminishing concentration of calcium. All of the patients excreted excessive amounts of calcium in the urine during the control periods. In the first three cases the diets were designed to ap-

proximate the customary intake of calcium and phosphorus. The urinary excretion of calcium by normal individuals taking the same diets varied from 0.15 gram to 0.70 gram per three-day period, with an average of 0.50 gram for eleven experiments. These values for normal calcinuria are consistent with reports in the literature (13, 35). The calcinuria of the patients with myeloma was unequivocally excessive. Although this is one of the metabolic findings which has been alleged to be of pathognomonic significance in the diagnosis of generalized osteitis fibrosa cystica, it is not infrequently observed in multiple myeloma (11, 13, 16, 27, 38). The urinary excretion of calcium by Case 4 with Paget's disease was strikingly in excess of that of normal individuals on low calcium diets. With an identical diet a control subject who exhibited no disturbances of calcium and phosphorus metabolism excreted 0.30 gram of calcium per three-day period in the urine (average of seven periods). The calcinuria of the control subject is comparable with that of several normal individuals in the series studied by Bauer, Albright and Aub (8), although it slightly exceeds the average of the series (0.19 gram per three-day period). Excessive calcinuria in Paget's disease is exceptional to the rule (34) but not without precedent (32) in the reported studies of the disease.

It has been noted that ingestion of extra phosphorus appears to have a very considerable effect on the urinary excretion of calcium (2, 13). In hyperparathyroidism the diminution of calcinuria during periods in which phosphate was fed has been construed to support Albright's theory of the functional pathology of that disease (*vide* Discussion). In Case 1 no definite association between calcinuria and intake of phosphorus can be inferred while in Case 2 the reduction of calcinuria is not large enough to be entirely convincing and may have occurred quite independently of the influence of the extra phosphorus ingested. The brevity of the studies necessitated by the seriousness of the patient's condition precludes any definite conclusions regarding the effectiveness of phosphate administration. The calcium in the urine of Case 4 strikingly and promptly fell when phosphate was added to the diet, and after a slight lag fecal calcium excretion likewise diminished. Thereafter the total calcium

balance was maintained within normal limits. Even when the intake of calcium was increased 0.50 gram daily by the ingestion of calcium lactate the calcinuria remained small. Unfortunately, the syndrome of transverse myelitis supervened during Period VI and the patient became incontinent of feces, but urine collections were complete up to the end of Period VII. Desired observations of the effects of withholding phosphate at this time were impossible because of the difficulty of collecting specimens. In the control subject who received the same diet as Case 4 the ingestion of phosphate resulted in a slight diminution of the calcinuria (0.30 gram to 0.16 gram per three-day period) which was reflected in a reduced negative calcium balance. In both subjects studied with a low calcium diet, Case 4 whose calcinuria was strikingly reduced by ingestion of phosphate and the control subject whose calcinuria at a lower basal level was only slightly diminished by the same amount of phosphate, the economy of calcium was effected without any significant change in the chemical pattern of the serum.

#### DISCUSSION

Hypercalcemia may and frequently does occur in pathological conditions when there is no anatomical evidence of an increase in the activity of the parathyroid tissue. In multiple myeloma it may or may not be associated with an increase in serum proteins. In most of the reported cases serum proteins are not recorded. The literature yields twenty-three instances of hypercalcemia in multiple myeloma where serum proteins were simultaneously determined. In fourteen of these, hyperproteinemia was observed (19, 22, 24, 27, 28, 33, 37) in four hypoproteinemia obtained (10, 13, 18, 19) and in five the total serum proteins were normal (10, 19, 23, 29, 39). Only four reports of the condition of the parathyroid glands at autopsy have been found in a series of over thirty case reports of multiple myeloma with hypercalcemia. Three of these (14, 24, 33) specify that the parathyroid glands were normal. The finding of 3 moderately enlarged glands in the case presented by Bulger et al. (13) is unique.

In view of the high concentration of proteins in the serum of Case 1 of the present report it is reasonable to assume that part of the total increase in the serum calcium was due to an increase



in the protein bound fraction. Herbert's (22) filtration studies with serum containing comparably elevated concentrations of protein and calcium (Patient A Ho) indicate that both diffusible and non-diffusible fractions of the serum calcium share in the total increase. In Case 3 the terminal elevation of serum calcium was not accompanied by any increase in the serum proteins; nevertheless both protein-bound and filtrable calcium were increased proportionately. When the serum contained 17.7 mgm. of calcium per 100 cc., 55 per cent of the total was ultrafiltrable, a figure which is consistent with the figures for percentage of diffusible calcium in normal sera under similar experimental conditions.

The mechanism of this elevation of serum calcium is quite unexplained. Comparable elevations of serum calcium are occasionally observed in cases of metastatic neoplasm involving bone. Five such cases have been studied in the New Haven Hospital in recent years; the serum proteins varied from 7.6 to 5.3 grams per cent and the serum calcium from 18.5 to 11.6 mgm. per cent, the lowest calcium concentrations occurring in the patients with the lowest serum proteins. Gutman (19) found serum calcium values greater than 11.0 mgm. per cent in 5 out of 26 cases. Mason and Warren (31) reported a case of metastatic breast cancer with persistent hypercalcemia varying between 17.6 and 12.9 mgm. per cent. The excretion of calcium on a low calcium diet was quite within normal limits. In their case a tumor in the neck, thought to be a parathyroid adenoma, proved to be a metastatic nodule from the mammary carcinoma.

There is apparently no correlation between the excessive calcium excretion in the urine and the concentrations of calcium in the sera. The same lack of relationship is obvious in the case (Case 3) reported by Bulger et al. (13). It would seem that the excretion of calcium by the kidneys depends on other factors besides the concentration of total calcium in the serum. The economy effected by the ingestion of phosphate without any change in the serum pattern is of especial significance in connection with theories which relate the effects of phosphate administration to the disturbed metabolism of hyperparathyroidism. Albright, Bauer, Clafin and Cockrill (2) studying the effects of phosphate ingestion in patients with

hypercalcemia, hypophosphatemia and excessive urinary excretion of both calcium and phosphorus<sup>2</sup> ascribed the diminution of calcinuria in the first two cases to the lower concentration of calcium in serum which allegedly resulted from the increased concentration of phosphorus. The findings in their third case were at variance with the concept of calcium as a threshold substance. During the first period of phosphate administration to Case 1 of Bulger et al. (13) the data accord with Albright's observations in his first two cases; but in subsequent periods of phosphate feeding, calcinuria diminished while serum calcium gradually increased and serum phosphorus dropped. The studies of Bulger's Case 3 present further evidence that administration of phosphate may affect calcemia and calcinuria quite independently. The mechanism whereby phosphate ingestion alters the urinary excretion of calcium is not clear, but certainly any hypothesis which relates the changes to alterations in the serum pattern fails to account for all the observed facts.

Knowledge regarding the physicochemical state of calcium in serum and the factors controlling its excretion is still fragmentary. That the parathyroid hormone plays a significant part in the regulation of calcium metabolism is clearly established. The significant question would seem to be whether forces other than an excess of parathyroid hormone can cause similar alterations in the metabolic functions. The three cases of myeloma and one of Paget's disease presented in this report constitute evidence that hypercalcemia and hypercalcinuria may occur in patients with normal parathyroid glands. While "secondary hyperparathyroidism" offers an attractive explanation for the hypercalcemia and hypercalcinuria in the rare and exceptional case in which hyperplasia of parathyroid tissue is observed (25) it seems improbable in cases where careful study reveals no anatomic evidence of hyperactivity. Granting all the imperfections in the present knowledge concerning the correlation of anatomic change and metabolic function, at least skepticism is justified

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<sup>2</sup> Parathyroid tumors were subsequently removed from the first two patients (Mr. C. M. and Mrs. N. B.), Cases 6 and 4; but the 3d patient, Mrs. M. D., is not included in the review of seventeen cases seen at Massachusetts General Hospital (1).

when hyperfunction is ascribed to glands which are grossly and microscopically normal.

#### SUMMARY AND CONCLUSIONS

Studies in three cases of multiple myeloma and one of Paget's disease exhibiting the alleged metabolic criteria of hyperparathyroidism are presented. The parathyroid glands in each case were normal. The excretion of calcium in the urine could not be correlated with its concentration in serum. Ingestion of phosphate diminished calcinuria without changing the chemical pattern of the serum.

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## APPENDIX

### *Protocols of Cases*

*Case 1*, J. B., a man of 60, was admitted to the New Haven Hospital July 18, 1930. In 1927 he had developed extreme weakness, anorexia and loss of weight which had lasted about a year. The relation of this to the condition for which he was finally admitted was not clear. In 1928 he had experienced nagging pain in the lower abdomen, and tarry stools. In June 1929 he became aware of increasing pains in the chest and back, weakness and loss of weight. The following October a number of teeth were extracted with subsequent intractable hemorrhage. In another hospital he was found to have a severe anemia with a high color index. Emaciation and dehydration were noted and there was tenderness over the costal cartilages and the rib margins and evident collapse of one vertebra. X-ray revealed marked decalcification of the lumbar vertebrae, while the skull, left femur and right forearm appeared normal. During December, pains in the back and ribs became more severe and extreme sacro-iliac pain developed. The patient survived an intercurrent pneumonia. The anemia did not improve in spite of intensive liver therapy. A course of parathyroid extract distinctly aggravated his symptoms.

On admission to the New Haven Hospital in July 1930, he was greatly emaciated and extremely weak. The blood pressure was 120/80. There was evident shortening and flattening of the lumbar spine and a severe anemia. X-ray showed decalcification of all the vertebrae and, to a lesser extent, of the ribs and pelvis, with slight rarefaction of the femora and skull, but none of the tibiae and fibulae. Three vertebrae had collapsed. The hemogram was typical of myelophthisic anemia. The urine contained a faint trace of albumin and frequent white blood cells and hyaline casts. Repeated tests for Bence-Jones protein in the urine were negative.

His general condition was desperate, feeding extremely difficult and he deteriorated with great rapidity. August 4th he had the first of a series of fractures of the ribs with steadily increasing deformity of the chest. January 21 he climbed out of bed in delirium and snapped his right femur. X-rays revealed increasing rarefaction of all bones, including the skull. During the last few days

of January, the patient became increasingly irrational, and incontinent of feces and urine. His blood pressure dropped to 100/70 before death which occurred February 3, 1931.

*Autopsy.* The neck and mediastinum were carefully searched for parathyroid tumor. All four of the small parathyroid glands which were removed showed normal microscopical structure.

*Anatomical diagnosis.* Diffuse myeloma involving the bone marrow of the vertebral bodies, ribs, sternum and femurs, with erosion of the bone cortex and metastases to lymph nodes, spleen, liver and kidney; anemia; emaciation; diffuse pneumonia (right lower); pulmonary arteritis with thrombosis; vegetative mitral endocarditis.

*Case 2*, A. C., a man of 52, was admitted to the New Haven Hospital December 7, 1933. Four months earlier he had begun to suffer from pain in the back and a month later had developed pains and tenderness in the ribs, accompanied by weakness and loss of weight.

On admission he appeared weak and emaciated. There was irregularity in the vertebral alignment, with tenderness over the spine and ribs. Several fusiform swellings were palpable in the ribs and a firm slightly tender mass was felt in the inferior portion of the right scapula. X-ray revealed rather general decalcification of the bones with widening of the medullary cavities and in addition focal areas of more intense rarefaction in many of the long bones, and collapse of at least one vertebra. The blood picture indicated a myelophthisic anemia. The urine contained large amounts of Bence-Jones protein (12 to 20 grams per day) without albumin. Biopsy of a rib confirmed the diagnosis of diffuse myelomatosis.

In the hope of checking or reversing the process of decalcification a parathyroidectomy was performed on January 5 by Doctor Samuel Harvey. Extensive search revealed three small parathyroid glands which were removed. No parathyroid tumor was found. The microscopic structure of the extirpated parathyroids was normal. (The patient developed pneumonia and died three days after operation. Permission for autopsy could not be obtained. The serum calcium just before death was found to be normal.)

*Case 3*, F. A., a man of 34, was admitted to the New Haven Hospital April 16, 1934. Six months earlier he had developed pain in his back after a slight wrench. This had gradually and steadily increased in intensity and extent until it involved the whole back and the ribs. X-ray on March 29 had revealed collapse of three lumbar vertebrae with decalcification of the spine, irregular decalcification of the pelvis and ribs, but nothing abnormal in the skull.

On admission his nutrition was fairly good but muscular weakness was evident and movements were guarded because of pain in the back. There was tenderness over the ribs and the lower dorsal and the lumbar spines. There was a moderate hyperchromic anemia and, as the condition progressed, increasing numbers of myelocytes

were found in the spread. The urine contained neither albumin nor Bence-Jones protein, and the formed elements of the sediment remained consistently normal. A biopsy of rib on April 17 confirmed the diagnosis of myeloma. The abnormal cells were identified as endothelial in type.

The patient deteriorated steadily, developing fractures of many ribs and of the pelvis and increasing deformities until his death which occurred on June 22, 1934.

At autopsy the neck and mediastinum were carefully dissected, but no parathyroid tumor was discovered. The microscopic structure of the small parathyroid glands which were found was normal.

*Case 4, W. C.,* a man of 64, was admitted to the New Haven Hospital December 19, 1932. For six or seven years he had suffered from pains in the back which were called lumbago. In August of 1932 he had had a more severe attack which had persisted, increasing in severity, until his admission, by which time he was confined almost entirely to a chair because of inability to stand upright or to lie down. The pains had radiated down the right sciatic nerve and had later involved the left leg. Shortly before admission he had noticed tender lumps on his head and pain in the right shoulder. Severe headaches had become a prominent symptom.

On admission his chief complaints were headache, pain about the right orbit, pain across the buttocks, down the legs and boring into the shins, and urinary troubles which he referred to an old stricture. He was markedly emaciated. There was tenderness over the spine and striking atrophy of the muscles of the legs. Numerous tender lumps were present over the skull and one on the left clavicle.

X-rays revealed the characteristic lesions of Paget's

disease in the spine, pelvis and femora. There was more than the usual rarefaction with patchy increases of density. There was moderate secondary anemia; no myelocytes were seen in the spreads. The urine contained albumin but no Bence-Jones protein.

The lumps in the skull increased steadily in size and new ones appeared over other bones. A tumor mass growing in the right orbit gradually extruded the eye from its socket. In the latter part of January he developed paralysis of both lower extremities and of the sphincters and soon after this signs of cranial nerve lesions appeared, presumably from the tumors of the skull. On February 8 pathological fracture of the right femur occurred. X-ray revealed that there was almost complete decalcification of the cortex of the upper half of the femur, the shadow cast being more like soft tissue than bone. Deterioration was rapid and death occurred on March 8, 1933.

*Autopsy.* Careful dissection of the neck and mediastinum disclosed parathyroid glands of normal size which on section proved to have normal microscopic structure. No parathyroid tumor was found.

*Anatomical diagnosis.* A. Nodular thickening, thinning and decalcification of bones of skull, femurs, tibiae, clavicles, sternum, ribs, scapulae, vertebrae and pelvis; a nodule in orbital plate with compression of the ocular bulb; bone nodules of pleura (left lower lobe); compression of spinal cord (5th thoracic vertebra); transverse myelitis; recent operation (suprapubic cystotomy); fracture of right femur; exophthalmos (right); splenomegaly. B. Generalized arteriosclerosis with especial involvement of coronary arteries; hydrothorax, hydropericardium.

*Subsidiary:* Fibrous pleural adhesions (bilateral); emphysema (bilateral). Clinically—Paget's disease.

# HEMOLYTIC ANTIBODIES FOR SHEEP AND OX ERYTHROCYTES IN INFECTIOUS MONONUCLEOSIS<sup>1</sup>

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Paul and Bunnell (1932) observed very high concentrations of agglutinins and hemolysins for sheep cells in the sera of patients during the acute stages of infectious mononucleosis, and this observation was found by these investigators to be of much value in the diagnosis of the disease. This finding has been confirmed and extended by Rosenthal and Wenkebach (1933), Boveri (1933), Bunnell (1933) and Bernstein (1934). In addition to the practical application in diagnosis, theoretical interest centers about the fact that antibodies for sheep cells are produced or enhanced in a disease of unknown etiology. Paul and Bunnell offered two explanations for their findings: (1) that the unknown agent responsible for infectious mononucleosis contains the heterophile or Forssman antigen; (2) that they were dealing with an example of isoagglutinin production elicited by abnormal cells, which are present either in the blood, or elsewhere, during active stages of the disease.

In studies of infectious mononucleosis by different investigators, a probable bacterial origin has often been suggested, with special mention of the streptococcus group, diphtheroid bacilli, and Vincent's organisms, all of which have been found in nose, mouth or throat lesions during the disease. Nyfeldt (1929) has reported the isolation of a small V-shaped or curved gram positive organism from the blood, which he called *Bacterium monocytogenes hominis*, and with which he produced the cellular blood picture of infectious mononucleosis in rabbits. Agglutination of this organism in 1 to 250 dilution by the serum of the patient was observed, but only five days after the temperature had become normal. Gorham, Smith and Hunt (1929) claimed to have produced the typical blood picture of the disease in guinea pigs by inoculation of membrane from the pharynx of

a severe case of Vincent's angina, while persons in contact with the experimental animals developed infectious mononucleosis. According to these investigators, heat-killed Vincent's spirochaetes also produced the blood picture in guinea pigs with subsequent immunity. The results mentioned above as to the etiology of infectious mononucleosis have not been confirmed.

The more recent studies on the antibody response in infectious mononucleosis seemed to us to offer a new angle of approach to the possible determination of the etiological agent and to an analysis of the properties of the blood serum in this disease. All the authors who have reported on the antibody response have used the terms heterophile or Forssman antibody in describing the sheep cells agglutinin and hemolysin in the sera of patients with infectious mononucleosis, on the assumption that the immune bodies are true Forssman antibodies merely because of their action on sheep cells. No results are recorded concerning absorption or other tests with various heterophile and non-heterophile antigens, such as guinea pig tissues and ox red cells respectively. In studies on the immunological properties and relationships of pneumococci and other organisms by Bailey and Shorb (1931, 1933a and b) and by Shorb and Bailey (1934), the observations by these workers and many others on the heterophile and non-heterophile antigens of various animal and bacterial species have been summarized. The results are given below.

Common or similar properties are often found in the normal and immune sera of man and many animal species and in the so-called heterogenetic antigens in certain animals and bacteria. The heterogenetic antigen most extensively studied was first observed by Forssman (1911) who found a substance in emulsions of the tissues of the guinea pig, cat and horse but not in the ox and rat, which stimulated in rabbits an hemolysin for sheep red corpuscles. The terms "heterologous," "heterogenetic," and "Forssman" have often been used in referring to these substances which have an antigenic

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factor in common with sheep red cells. It is known, however, that sheep red cells contain at least two and probably more distinct antigens; one of them in common with ox red cells is thermolabile and gives rise to a species or group specific hemolysin in rabbits, the other resists boiling, and stimulates in rabbits an hemolysin which is not species or group specific. To the former Friedemann (1917) applied the term "isophile" and to the latter the name "heterophile" (i.e., having an affinity for the receptors of other and phylogenetically unrelated species). These terms are better chosen since they can be applied both to the antigen and antibody while the term heterogenous refers only to the ability of the antigen to engender antibodies for such unrelated species.

A large number of animals and bacteria possess chemical and immunological properties practically indistinguishable from the thermostable substance found in sheep red cells. The animals having heterophile antigen in their tissues or blood are said to belong to the "guinea pig type" and those not having it are classed as the "rabbit type." Alcoholic extracts of heterophile antigens are capable of inhibiting *in vitro* the action of heterophile hemolysins when mixed with them, of fixing complement in their presence and of precipitating these antibodies; but such extracts act only in this way as "haptens" because they are incapable of stimulating the production of hemolysin *in vivo* unless combined with a protein or other suitable colloid. Furthermore, heterophile antibodies can be removed from solution by absorption with heterophile antigens; they generally have the property of primary toxicity for the guinea pig; and in many cases they will agglutinate as well as hemolyze sheep red blood cells, although the ratio of hemolysins and agglutinins may vary greatly. Heterophile antibodies can be produced only in animals of the rabbit type, while isophile antibodies can be produced in both the rabbit and guinea pig types.

It is well known that an immune serum from the rabbit, obtained by injecting ox red corpuscles, contains a certain amount of hemolysin for the sheep's red corpuscles as well as for those of the ox; the same is true for both species of cells if sheep red cells are injected instead of ox cells. These hemolysins are engendered by the thermolabile isophile or group antigens of these zoologically related species. It is known too that ox red cells also possess a thermostable substance which has, however, entirely different antigenic properties from the ordinary thermostable Forssman component of sheep red cells. Whether ox cells and sheep cells have identical or similar thermostable factors in common has not been determined. It has been shown by Landsteiner and van der Scheer (1925) by means of the flocculation reaction, that similar substances or common factors exist even in the blood of many distantly related species, which can be extracted from the blood with alcohol. It was further shown that in some instances an immune serum may not be markedly hemolytic for a certain blood and still contain an antibody active upon a fraction thereof, as in the case of the flocculation of anti-horse sera by rat blood

extracts. Such reactions are not true heterophile reactions of the Forssman type, but they are heterogenous in the broadest sense of this term.

The blood of human beings and rabbits normally contains heterophile (Forssman) hemolysin of low titer for the red corpuscles of the sheep, but no isophile hemolysin is found naturally in the serum of either of these species. In human beings with certain clinical conditions antibodies for sheep red cells have been shown to be greatly increased in amounts and to be of definite value in diagnosis. Thus, Davidsohn (1929, 1930) has demonstrated a marked rise in the titers of lysins and agglutinins for sheep red cells (and a slight increase for the red cells of the ox, guinea pig and rabbit) in the sera of patients who had been injected with horse serum, and that the average titers for these antibodies were very much higher among those patients who subsequently developed serum sickness than among those who did not develop allergic symptoms. Since horse serum contains some heterophile antigen it was to be expected that the antibodies for sheep cells were mostly of the Forssman type. This was shown to be the case by Ramsdell and Davidsohn (1930) and Davidsohn and Ramsdell (1929, 1930) by means of absorption tests with known heterophile antigens, such as guinea pig tissues, and by the increased primary toxicity of the sera for this animal. Similar results were found by Bailey and Shorb (1931) who noted an increase in the hemolytic titers of the sera of patients who were recovering from lobar pneumonia, a disease caused by the pneumococcus, all types of which were shown by them to contain the Forssman antigen.

With these observations in mind we have made the present study with the object of determining more definitely the serological properties and relationships of the hemolytic and agglutinative antibodies in infectious mononucleosis. The results of this study comprise the present report.

#### METHODS

**Serum titrations.** The sera were titrated both for agglutination and hemolysis of the various cells used. In the agglutination tests, 1.0 cc. of the various dilutions of inactivated serum plus 0.5 cc. of a two per cent suspension of washed erythrocytes were used in each tube. This was incubated in the water bath at 37.5° C. for one hour, placed in the icebox over night, and then read for macroscopic agglutination. The highest dilutions showing a complete (4+) and a definite (+) positive reaction were recorded. Tubes showing  $\pm$  agglutination were not regarded as significant.

Hemolytic titrations were carried out in two ways. In the absorption method, the same sets which had been read for agglutinins were used. After recording these values, the supernatant sera were drawn off the cells and 1.0 cc. of isotonic saline was added to each tube, followed by 0.2 cc. of pooled fresh guinea pig serum diluted 1 to 10.



The racks were well shaken, incubated for one hour at 37.5° C. in the water bath, and read for hemolysis. The second method was the direct titration of the serum. To the dilutions of serum in 1.0 cc. volumes was added 0.5 cc. of two per cent red cell suspension, followed by the same guinea pig complement. The tubes were shaken and incubated at 37.5° C. in the water bath for one hour. The absorption method was favored because it eliminated the possibility of anticomplementary action of the serum. Both methods were used as checks on each sample of serum. The titer of hemolysins was determined from the highest dilution of serum showing complete hemolysis. The last tube showing definite (+) lysis was also recorded.

It is to be noted that in these tests 1.0 cc. of serum dilution was used in each tube, so that the titers recorded indicate the number of units of agglutinin or hemolysin per cubic centimeter of the original undiluted serum. Attention is brought to this because previous authors (Paul and Bunnell (1932), Bunnell (1933), Boveri (1933), Bernstein (1934)) who have reported on infectious mononucleosis have followed the technic of Davidsohn (1929), in which 0.5 cc. of each serum dilution was used. For purposes of comparison the titers indicated in this paper are therefore actually double those which would be recorded by Davidsohn's method.

*Absorption tests.* These tests were carried out with a variety of heterophile and other antigens. The method used was essentially that employed by Bailey and Shorb (1931, 1933b), and is briefly as follows:

The serum to be absorbed was diluted so that 1.0 cc. contained a convenient number of units of antibody, usually forty or eighty. About 0.2 to 0.4 cc. of the finely divided absorbing antigen was packed by rapid centrifugation and the supernatant saline was carefully drawn off completely. To the wet absorbing antigen in the centrifuge tube, 1.0 cc. of the diluted serum was added, the two thoroughly mixed, and incubated in the water bath at 37.5° C. for two hours. The mixture was then centrifuged at high speed for a few minutes and 0.5 cc. of the supernatant fluid was drawn off and diluted in a series of serological tubes. One unit of the red cells against which the serum was to be titrated was added to each tube and the incubation again carried out at 37.5° C. for one hour. The set was then placed in the icebox over night, and the next morning was read for agglutinins. The supernatant fluids were now pipetted off and 1.0 cc. of salt solution was added to each tube for resuspension of the cells. Complement was added and the tubes were again incubated at 37.5° C. for one hour and then read for hemolysis. Control series in which the sera were not absorbed, but were otherwise treated similarly to the absorbed samples, were always run for comparison. In determining the extent of absorption of agglutinins or hemolysins, the test sets were compared with the controls.

The number of units of antibody used in each test varied between 40 and 160, though 40 or 80 units were used in most instances. Because of this variation, however, absorption values in the tables are given in terms

of per cent of total antibodies absorbed, rather than as absolute figures.

The absorbing antigens used were either tissues, bacteria or erythrocytes. The tissues were finely ground, and 0.2 to 0.4 cc. of the packed material was used for each test, depending on the number of units of antibody to be absorbed. The bacteria were grown in 0.5 per cent glucose beef infusion broth (with vaseline seals for the anaerobes) for as long a period as necessary to obtain a heavy growth, usually 24 hours. The packed bacteria of 50 or 100 cc. of broth culture were used in each test, depending on the units of antibody to be absorbed. The erythrocytes were used in the ratio of 10 units of packed cells for each 40 units of antibody to be absorbed. Bailey and Shorb (1933b) have shown that 10 units of boiled sheep red cells may remove from 760 to 930 units of hemolysin from heterophile rabbit sera. The details of treatment of the antigens (heating, etc.) are indicated in the absorption tables.

*Neutralization tests.* Tests were carried out with salt solution emulsions of alcoholic extracts of various tissues and bacterial and blood cells. It is well known that such emulsions of the lipoids of heterophile antigens combine with the corresponding antibody. The extracts were employed in order to determine the possibility of their interference with the sensitization of the red cells by the infectious mononucleosis antibodies. The preparation of the extracts is described by Bailey and Shorb (1933a and b). The alcoholic emulsions were made by the slow addition, with shaking, of 4.5 cc. of saline to 0.5 cc. of extract. Fairly opalescent emulsions were obtained in this manner. All the extracts of Forssman antigens used here have been repeatedly tested for their ability to neutralize heterophile hemolysins and found effective. These tests were carried out in essentially the same manner as the absorption tests. Serial dilutions of the serum were put in serological tubes, 0.5 cc. of lipid emulsion was added to each tube, and the mixtures were incubated for two hours in the water bath at 37.5° C. One unit of red cells was then added to each tube. From this point onward the technic was the same as that used in the absorption tests.

#### CLINICAL ASPECTS OF CASES REPORTED

This report deals primarily with the immunological findings in three clinically and serologically definite cases of infectious mononucleosis. Evidence is offered which supports the conclusion that the antibodies in this disease are not heterophile or Forssman immune bodies, a fact which may be of value in the diagnosis of this condition as well as in the explanation of certain phenomena which occur in the disease. The clinical data in these cases are given below and the temperature curves and blood pictures are illustrated in Figures 1, 2 and 3.

Case 1.<sup>2</sup> F. C., a white, male, medical student, aged 22 years, was admitted to The Johns Hopkins Hospital on November 28, 1933, complaining of malaise and severe headache of three days duration. The temperature on admission was 101.5° F.

Examination at the time of admission showed nothing striking. There was no gingivitis. A vestigial remnant of tonsil on the right side was moderately hyperemic, as was the pharynx. There was a leukopenia at this time, the white cell count being 3,250. The differential count was normal, however, nor were any cellular abnormalities noted. Within the next week, changes occurred which pointed to the correct diagnosis. There was occa-

completely recovered and was discharged from the hospital.

Blood culture was negative on the third day of hospitalization. On the fifth day a culture of the throat revealed *Staph. aureus* and *albus*, and an alpha hemolytic streptococcus.

The blood serum on the fifth day agglutinated *B. typhosus*, *B. paratyphosus B* and *B. suispestifer I* all in dilutions of 1 to 160. It should be noted that the patient had received typhoid vaccine in 1927. Three days later the bacterial agglutinations were: *B. typhosus*, 1 to 80; *B. paratyphosus A*, 1 to 20; and *B. paratyphosus B*, 1 to 320.

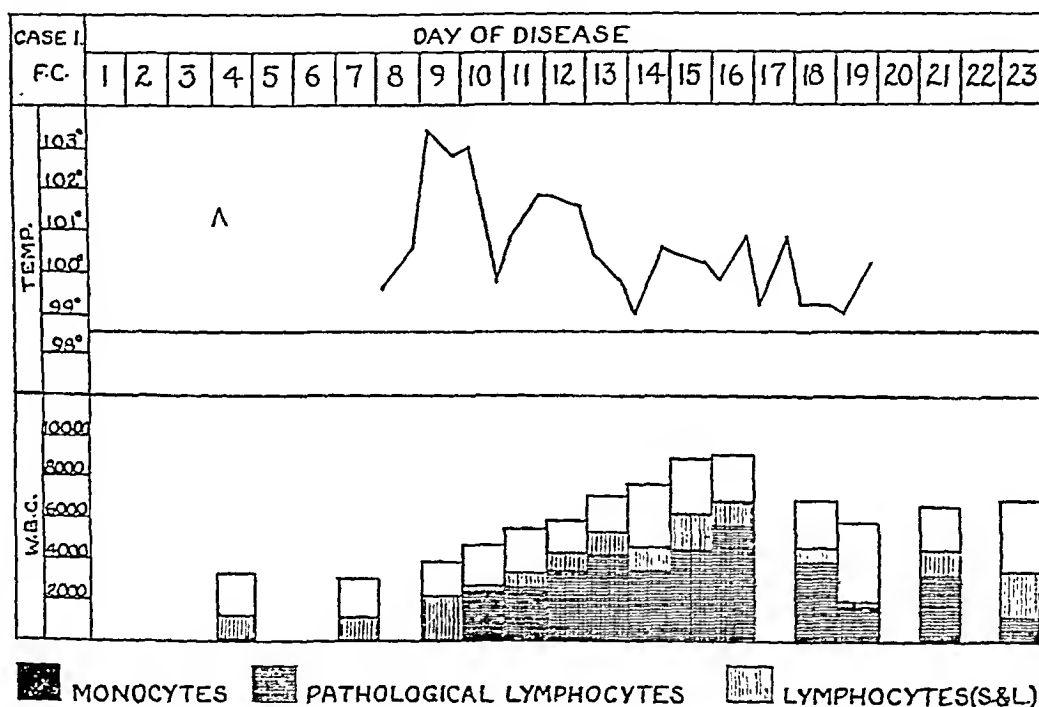


FIG. 1. CASE 1. INFECTIOUS MONONUCLEOSIS.

sional nausea, and on one occasion, spontaneous epistaxis. The throat was injected but only slightly sore. The cervical glands and the spleen became definitely palpable. At this time the abnormal lymphocytes characteristic of infectious mononucleosis first appeared, and the serum on December 4 agglutinated sheep erythrocytes in 1 to 64 dilution. Symptoms continued for 10 days longer. The temperature course and blood findings are shown in Figure 1. On the twenty-second day the patient had

Agglutination of sheep erythrocytes occurred in 1 to 64 dilution when first tested for on the sixth day after admission, the same day on which the characteristic symptoms and pathological blood cells of the disease were first noted. Two days later the agglutinins had risen to 1 to 256, and six days after this they reacted in a dilution of 1 to 4,096.

For experimental purposes, aseptically collected blood and nose and throat swabs were obtained on December 13, fifteen days after admission, at a time when the patient had a rather severe sore throat.

The serum was separated from the clot, the latter was macerated in a sterile mortar, broth was

<sup>2</sup> The authors are indebted to Dr. Alan Bernstein of The Johns Hopkins Hospital for clinical data and blood samples from this patient. The case was reported as Case 14 in the series presented by Dr. Bernstein in his paper, "Antibody Responses in Infectious Mononucleosis," J. Clin. Invest., 1934, 13, 419-435.



added as a diluent, and it was inoculated into agar and rabbit blood broth tubes, as well as into a rabbit, a guinea pig, a rat, and a mouse. The tubes remained sterile, nor did any of the animals show any indication of an infectious process.

Culture of the nose and throat swabs revealed *Staph. albus*, and alpha and gamma hemolytic streptococci. Part of the nose and throat swab broth washings were injected intraperitoneally into a rabbit, guinea pig, rat and mouse. No visible reactions occurred in the first three animals, but the mouse died within 96 hours. Gram positive diplococci and short chains were found in pure form in smears from the heart blood and peritoneal and thoracic cavities. These were iso-

Case 2. N. H., a white, male, graduate student, aged 22 years, was seen on July 11, 1934, at which time the complaint included a severe soreness of the throat and gums and general adenopathy. During the preceding two weeks the patient had noted a fatigued feeling and frequent headaches on arising in the morning, which gradually subsided during the course of the day. Five days before, the soreness of the throat and gums had set in, and at this time enlargement of the cervical glands in the anterior triangle on the right side was first noticed by the patient. These symptoms had become progressively more severe to the time of examination.

At the time the patient was first seen, the temperature was 100° F. The right anterior cervical and right inguinal glands were enlarged and tender, and the axillary nodes also were slightly palpable. The spleen and liver could not be felt. The soft palate was congested and bore an area of ulceration about one centimeter in diame-

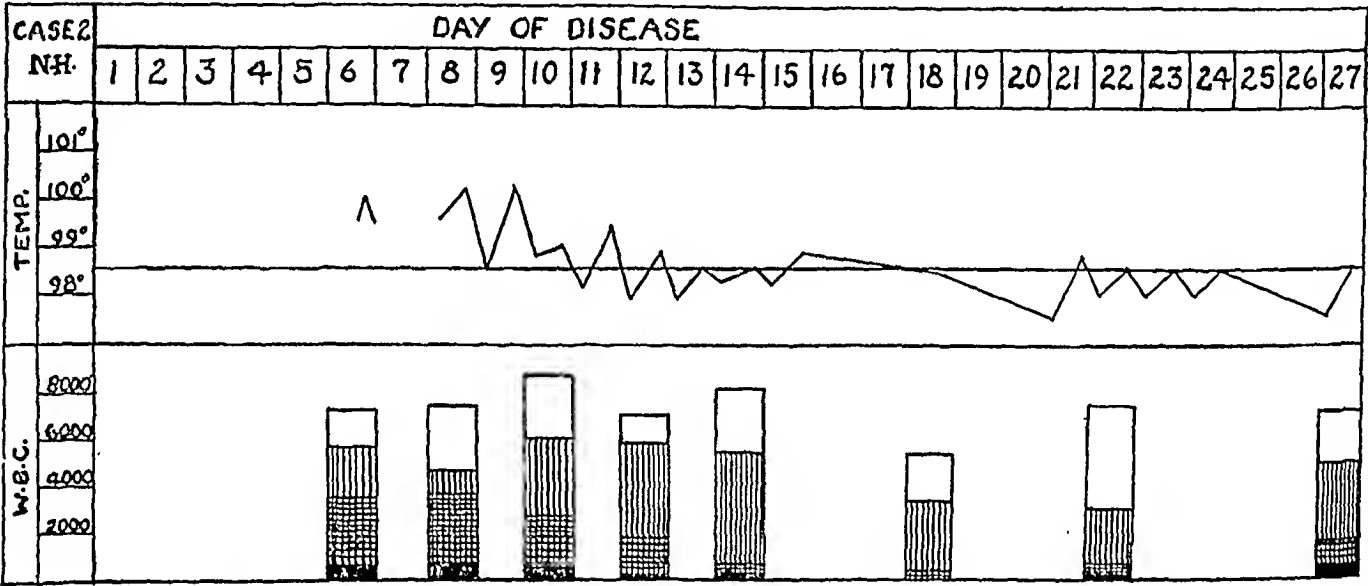


FIG. 2. CASE 2. INFECTIOUS MONONUCLEOSIS.  
Legends as in Figure 1.

lated on rabbit blood agar and proved to be gamma streptococci.

Nose and throat cultures were again made on the day of discharge from the hospital, when the patient had completely recovered from symptoms. At this time only a hemolytic staphylococcus (*albus*) was recovered.

All the organisms thus isolated were used for attempted absorption of antibodies from the patient's serum, in an effort to establish a possible etiological relationship to the disease. The results of these tests are given in a subsequent section.

The second case showing the clinical and serological features of the disease is reported below.

ter which was covered with a white diphtheritic membrane. A culture of this was negative for *B. diphtheriae*. The total leukocyte count at this time was normal and remained within normal limits during the subsequent course of the illness. The differential, however, showed 72 per cent lymphocytes, of which 41 per cent were the pathological forms typical of infectious mononucleosis. The temperature course and additional leukocyte studies are shown in Figure 2. Agglutination of sheep cells in a dilution of 1 to 1,024 with serum obtained on July 13 confirmed the diagnosis.

Subsidence of symptoms and recession of the lymph glands began after six days, though a "weak" feeling of the gums continued. These were not markedly inflamed, but on the right side they showed the beginning of an ulcerative stomatitis which was found to harbor a profusion of spirochaetes and fusiform bacilli. Within a week this had developed into a severe Vincent's angina. This

yielded to specific treatment, whereupon complete recovery ensued.

Bacterial agglutinations with serum collected on July 18 were negative in dilutions of 1 to 20 with *B. typhosus*, *B. paratyphosus A*, *B. paratyphosus B*, *B. suispestifer*, *B. abortus*, and *B. proteus X 19*, but a test with *B. melitensis* was positive in 1 to 80 dilution.

Six days after the patient was first seen, a swab of the soft palate on and around the membrane showed *Staph. albus* and a gamma streptococcus. These were used for absorption of the serum, as reported further on.

It is of interest that this patient was kept at home during the entire course of his illness, with no precautionary measures as to contact with other members of the family. Several of these were within the age range for infectious mononucleosis (between 18 and 25 years), yet no contagiousness nor infectiousness by ordinary contacts was apparent. Gorham, Smith and Hunt (1929) have reported transmission of the disease between experimental animals and laboratory workers in contact with them, as well as between human beings.

The course of the third case is presented below.

**Case 3.<sup>3</sup>** F. W., a white, female, nurse, aged 36 years, was admitted to the Sinai Hospital on September 18, 1934, complaining of pain in the back on both sides, enlarged glands, sore throat, and very severe headache.

Eight days before admission, the patient suffered severe pains in the right and lower regions of the back. The pain was intermittent and radiated toward the umbilicus, but not to the right shoulder or scapula. At this time, she noticed also that her cervical, axillary and inguinal glands were enlarged and tender. Coincident with this there was a constant dull headache in the frontal and parietal areas. Four days later the pain in the lower back extended to the left side and this continued up to the day before admission. During this period the patient had been nauseated constantly and had noted that her stools were very light in color. She now became aware of the fact that her skin had assumed a light yellow tinge. At the same time she began to feel chilly and feverish. By the day of admission the jaundice had to a great extent subsided.

The morning of the day before admission the patient vomited large white curds after drinking milk, and during the remainder of the day suffered a dull pain in the epigastrium, which was no longer present at the time of examination. On the same day her throat became very sore.

On admission, the temperature was 101° F. There was an icteric tint to the sclerotics, the pharynx was granular, and the pillars inflamed. The cervical, axillary and inguinal nodes were enlarged. The liver and spleen were not palpable. The icteric index was 50, and immediate biphasic van den Bergh reactions were obtained. The total leukocyte count at this time was 9,700, with 80 per cent lymphocytes, most of which were of the characteristically abnormal type. The subsequent temperature course and differential blood pictures are illustrated in Figure 3.

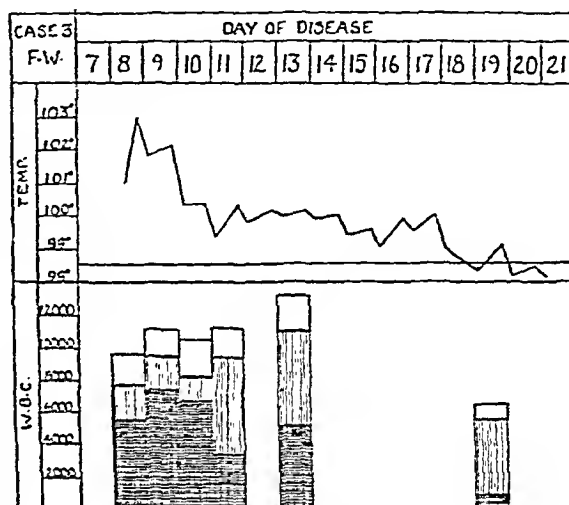


FIG. 3. CASE 3. INFECTIOUS MONONUCLEOSIS.

Legends as in Figure 1.

After four days, the symptoms diminished in severity, and the glandular enlargement became less pronounced. The sclerotics were still slightly icteric, but x-ray revealed no gallbladder pathology. On the fourteenth day the patient was discharged from the hospital.

The Wassermann reaction on September 19 was negative. On September 21 the serum agglutinated sheep erythrocytes through a dilution of 1 to 512, which was the lowest concentration of serum tested. The next day definite agglutination of these cells was found in 1 to 4,096.

Throat culture on admission revealed a hemolytic staphylococcus. A blood culture made the next day was negative.

On September 22, at the time when the acute symptoms had begun to subside, blood was obtained aseptically and defibrinated. Within 30 minutes of collection, the whole blood was transferred to the following media: (1) patient's blood incorporated in beef infusion agar, (2) patient's blood in beef infusion broth, (3) normal human erythrocyte beef infusion agar, (4) normal hu-

<sup>3</sup> The authors wish to express their thanks to Dr. L. F. Krumrein and Dr. L. Katzenstein of the Sinai Hospital, Baltimore, for the clinical data and blood of this patient.

man erythrocytes in small amount of normal plasma, (5) rabbit blood beef infusion agar, (6) rabbit blood beef infusion broth, (7) chocolate rabbit blood beef infusion agar, (8) ox spleen infusion broth, (9) ox lung infusion broth (with and without glucose, both aerobic and anaerobic), (10) ox brain infusion broth (with and without glucose, both aerobic and anaerobic), (11) ox liver infusion broth, and (12) sheep hemoglobin infusion agar.

After prolonged incubation at 37.5° C., no growth was found in these tubes by staining methods, though certain of them were suggestively turbid. The contents of the turbid tubes, together with a small amount of the patient's defibrinated blood, were injected intravenously into rabbits. These developed hemolysins for sheep cells in titers as high as 1 to 1,660 on the seventh day after the last injection. Further studies on this point are now being carried on, the results of which will be reported in a later paper.<sup>4</sup>

### Serology

**Titration.** In Table I are tabulated the titers of sera collected at intervals during the active stages of illness in the three cases described. In Cases 1 and 2, results obtained with serum collected some time after recovery are also shown. Titrations were carried out against sheep and ox red cells.

The parallelism of agglutinin and hemolysin values for sheep erythrocytes in the sera obtained during the illness is striking in all instances except with the first sample in Case 2 where the end-point of hemolysis was considerably lower than that for agglutination. In Case 3 also the end-points are considerably separated, though the difference here indicates a variation of only one tube in the series of serum dilutions. The values of complete agglutination and hemolysis in this instance are quite close. Paul and Bunnell have previously discussed the parallelism of the antibodies in this disease.

In Case 2 the range of complete hemolysis of sheep cells is not higher than that which may be infrequently found in normal human sera, accord-

TABLE I

*Agglutinins and hemolysins for sheep and ox red cells in the sera of cases of infectious mononucleosis\**

Serum collected	Sheep red cells— titers				Ox red cells— titers			
	Agglu- tinins		Hemo- lysins		Agglutinins		Hemo- lysins	
	4+	+	4+	+	4+	+	4+	+
Case 1—F. C.								
December 12, 1933..	500	1,170	500	1,225	0	0	1,280	7,680
December 19, 1933..	480	1,920	500	1,625	0	0	1,000	2,830
March 20, 1934†....	0	0	40	84	0	20	15	40
Case 2—N. H.								
July 13, 1934.....		1,024	128	400	0	0		
July 16, 1934.....		500	100	500	0	0	128	1,250
July 18, 1934.....		350	70	230	0	0	114	1,000
September 17, 1934†		8	8	24	0	8	8	32
Case 3—F. W.								
September 22, 1934..	512	4,096	384	2,048	32†	1,024†	1,536	6,400

\* The agglutinin titers reported by previous authors (Paul and Bunnell, Boveri, Bunnell, Bernstein) were given in terms of the highest dilutions of serum showing definite (+ or ±) macroscopic agglutination. In this table complete and definite reactions are both recorded in order to better demonstrate the parallelism of agglutinins and hemolysins for sheep red cells.

† Sera obtained some time after complete clinical recovery.

‡ Cells easily stirred up in all tubes—definite but loose agglutination.

ing to the values given by Bailey and Shorb (1933b) and by Davidsohn (1929) who found titers between 128 and 256<sup>5</sup> in 2.1 per cent of 379 persons tested. Normal agglutinins are much lower, however, for in a series of 450 normal adult human beings reported by Davidsohn (1930), 19 cases (4.2 per cent) showed agglutinins and the average titer of these antibodies was 8.

As shown in the table, the hemolysins for ox erythrocytes were found to be quite high and present in greater concentration than the sheep cells hemolysins, though appreciable agglutination of these cells was found only with the serum of Case 3. Unlike the sheep cells agglutinated by sera from these cases, the ox cells were very easily shaken loose even in the tubes containing the greatest concentrations of serum. A comparison with the titers found in a small series of nor-

<sup>4</sup> "Attempts at the Experimental Production of Infectious Mononucleosis Antibodies in Rabbits." (Unpublished data.)

<sup>5</sup> Values "corrected" to conform to our expression of titer as the units of antibody per cubic centimeter of serum.

mal sera is afforded by Table II. Eighteen of twenty sera tested showed agglutinins, 10 being the average titer. Complete hemolysis in dilution of 1 to 4 or above was shown only by two sera, while definite hemolysis, found in nineteen cases, showed an average  $\pm$  titer of only 8. Davidsohn (1930) has reported a lower incidence of ox cell agglutinins in normal sera than shown here. According to this investigator they occurred in 25 per cent of 55 persons. The average titer of 8 which he found was of the same degree of magnitude as reported in Table II.

TABLE II

*Agglutinins and hemolysins for ox red cells in normal human sera*

Serum number	Ox red cells—titers		
	Agglutinins	Hemolysins	
	$\pm$	4 $\pm$	$\pm$
1.....	8	0	10
2.....	0	0	10
3.....	0	10	20
4.....	8	0	2
5.....	32	0	24
6.....	8	0	6
7.....	16	0	2
8.....	16	0	8
9.....	16	0	8
10.....	4	0	6
11.....	8	0	2
12.....	8	0	2
13.....	4	0	6
14.....	8	4	12
15.....	8	0	4
16.....	8	0	4
17.....	8	0	4
18.....	16	0	8
19.....	4	0	4
20.....	4	0	0
Average titer of positives.....	10	$\pm$	8

It is evident then, that ox cell antibodies in the form of lysins are present in high concentration in infectious mononucleosis, and that these disappear with recovery. The agglutinins are apparently increased only in certain cases, and to a limited extent. Thus, in one instance these were below the normal average value, as illustrated by the relative titers during and after illness in Case 2. An increase of ox cell antibody titer in human beings has been reported in but one circumstance of which we are aware; following serum therapy. Davidsohn (1930) reported an increase of agglutinins for ox cells, among others, following injec-

tions of horse serum, but this did not occur so regularly nor to such an extent as the increase of agglutinins for sheep cells. In most patients who did not have them before injection, they appeared after the onset of serum sickness. Deicher (1926) had previously found, in a series of patients treated with horse and sheep serum, definite agglutinins for sheep and ox cells as well as a variety of other erythrocytes. Davidsohn (1929) considers that the increase of antibodies for cells other than those of the sheep may be due to a non-specific stimulation, since such immune bodies are already normally present in human serum. This author does not mention what titers were obtained with the ox cells, but his discussion leaves the impression that they were quite low.

The presence of ox as well as sheep cell antibodies in this disease did not of itself, of course, indicate necessarily that the antibodies for sheep cells were not heterophile in nature. The possibilities were naturally considered that either the antibodies for the two types of cells coexist as responses to an etiological factor containing antigenic components in common with both types of erythrocytes, or that one or the other, or both were the result of non-specific stimulation. This latter view seems hardly probable for reasons which will be discussed further on. A number of experiments given below demonstrate the true nature of the antibodies found in this disease.

*Absorption tests.* The samples of serum of which the titers are recorded in Table I were used in all tests done. Table III illustrates the results of absorption tests carried out with sera obtained during the courses of illness in the three cases. Only the more important or doubtful tests were repeated with the sera of Cases 2 and 3. For convenience of discussion the tests are separated into groups.

In the first group of tests carried out, sheep and ox erythrocytes were used for absorption. It is immediately evident that the antibody can be absorbed by either of these cells for themselves and each other. Since the antibodies against sheep cells are absorbed by ox cells, and ox cells do not contain heterophile antigen, it is apparent that these are not heterophile antibodies.<sup>6</sup> The anti-

<sup>6</sup> After completion of this paper, a communication by Stuart, Burgess, Lawson and Wellman (Arch. Int. Med.,

bodies are a response to an antigenic component which these cells have in common. In the introduction a discussion has been given of the so-called isophile antigen, a *thermolabile* substance common to both sheep and ox erythrocytes. Here, however, it is seen that boiled and autoclaved corpuscles of both species are as effective as the fresh cells in absorbing antibody. The conclusions which may be drawn from this are either (1) the antigenic component concerned in this case is not isophile but some unknown antigen common to ox and sheep cells which is heat stable, or (2) that heating destroys the antigenic power of isophile antigen so far as its ability to stimulate antibodies is concerned, but leaves a heat stable hapten which possesses the power to absorb such antibodies. We are not aware that work has been done to demonstrate the presence of such a hapten in the isophile antigen. In a later report we shall discuss the results of attempts to establish the antigenic relationship of the heated corpuscles of the two species to the isophile component of these cells. It may be stated here that the isophile antigen has been found to contain no thermostable hapten, and that it apparently bears no relationship to the heat stable component responsible for absorption of mononucleosis antibodies.

It is interesting to note that the antigen in the ox erythrocytes is broader than that in the sheep cells, for the former removes antibodies against sheep cells to a greater degree than the sheep cells absorb ox hemolysins. In Cases 2 and 3 in particular the sheep cells do not completely remove antibodies even against themselves; as a matter of fact, these agglutinins are removed better by the heterologous ox corpuscles. An explanation for this is difficult to conceive, unless it is that the sheep antigen-antibody combination is a loose one, allowing some dissociation during the manipulations involved in the absorption test.

It may be stated that on the basis of the results of previous investigations as well as our own

work, antibodies of the type seen here are not found in normal human serum. That the antibodies for sheep cells in such serum are heterophile is well known and has been recently demonstrated with bacteria by Bailey and Shorb (1933b) and Shorb and Bailey (1934), who found that all natural human anti-sheep hemolysins were absorbed by *B. lepi-septicus* 370, pneumococcus, *Strep. viridans*, *M. catarrhalis*, and partially by *B. anthracis*, *B. faecalis alcaligenes*, *B. paratyphosus* B and *B. aertrycke*. These bacteria contain heterophile antigen. We have found, however, that such normal antibodies are not absorbed by ox erythrocytes. It has been discussed before that immune bodies for ox cells are found in normal human blood. Davidsohn (1930) has shown that they are not related to the presence of sheep cell antibodies, for in a group of 55 normal individuals, of those sera having no agglutinins for ox blood, almost one-half had agglutinins for sheep blood. The ox cell antibodies are increased in serum sickness, as shown by Deicher (1926) and Davidsohn (1930). The former author found that these are absorbed only by the homologous cells.

In two of the three cases with which this report deals, samples of serum were obtained some time after complete recovery from the illness. The essentially normal titers of these sera for ox and sheep corpuscles are shown in Table I. Absorption tests were carried out with both these sera. In Case 1 the sample was taken three and one-half months after recovery. Tests showed that whereas the antibodies for sheep cells were completely removed by carp gill suspension (heterophile tissue), fresh and autoclaved ox cells did not absorb them to the slightest degree. This seems to us to be of especial significance for this person had comparatively recently been subjected to the stimulatory factor responsible for such antibodies. The serum of Case 2 was obtained not quite two months after recovery, and though the antibodies were found to be low for sheep and ox cells, they were not all of the heterophile type for the former cells. Absorption with carp gill removed about 60 per cent of sheep hemolysins, whereas ox red cells took out about 40 per cent of these hemolysins. Apparently this determination was made at a time when the infectious mononucleosis antibodies had almost completely dis-

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1934, 54, 199-214) appeared, dealing with the cytological and serological aspects of infectious mononucleosis. The authors found that the antibodies were not absorbed by guinea pig kidney or testicle, and conclude that "certain facts must be explained before the heterophile nature of the antibody in mononucleosis can be unreservedly accepted."

TABLE III

Absorption tests with various heterophile and non-heterophile antigens and the sera of cases of infectious mononucleosis

Group number	Absorbing antigen	Serum Case 1—F. C.			Serum Case 2—N. H.			Serum Case 3—F. W.			
		Per cent units absorbed			Per cent units absorbed			Per cent units absorbed			
		Ox		Sheep	Ox		Sheep	Ox		Sheep	
		Hemo-lysins	Agglu- tinins	Hemo- lysins	Hemo- lysins	Agglu- tinins	Hemo- lysins	Agglu- tinins	Hemo- lysins	Agglu- tinins	Hemo- lysins
I	Sheep red cells (fresh) . . . . .	85	100	100	56	62	90				
	Sheep red cells (boiled 2 hours) . . . . .		100	100							
	Sheep red cells (autoclaved $\frac{1}{2}$ hour) . . . . .	87.5	100	100	70	75	90	50-75	93	97	100
	Ox red cells (fresh) . . . . .		100	94	100	100	81				
	Ox red cells (boiled 2 hours) . . . . .		100	96							
	Ox red cells (autoclaved $\frac{1}{2}$ hour) . . . . .	100	100	94	100	100	86	100	100	100	97-99
II	Guinea pig kidney (boiled) . . . . .		0	0							
	Guinea pig heart (fresh) . . . . .		0	0							
	Carp roe* . . . . .		0	0							
	Carp gill* . . . . .		0	0							
	Terrapin* . . . . .		0	0							
	Mouse* . . . . .		0	0							
	Dog kidney* . . . . .		0	0							
	Cat kidney* . . . . .		0	0		0	0				
	Chicken red cells (fresh) . . . . .		0	0	0						
	Chicken red cells* . . . . .		0	0							
	Horse kidney* . . . . .				0	43	45	75	0	50	0
	<i>B. lepi-septicus</i> 370† . . . . .		0	0		0	0				
	<i>Diplo. pneumoniae</i> I DRI† . . . . .		0	0							
	<i>M. catarrhalis</i> E (human)† . . . . .		0	0							
	<i>Streptococcus</i> ( <i>Viridans</i> group HT)† . . . . .		0	0							
	<i>Streptococcus</i> ( <i>Viridans</i> group Cl)† . . . . .		0	0							
	<i>B. faecalis alcaligenes</i> E† . . . . .		0	0							
	<i>B. alcaligenes bookeri</i> L† . . . . .		0	0		0	0				
	<i>B. megatherium</i> F 19A† . . . . .		0	0							
	<i>B. cereus</i> F 13A† . . . . .		0	0							
	<i>B. anthracis</i> Pg 208B (autoclaved) . . . . .		0	0							
	<i>B. dysenteriae</i> Shiga L† . . . . .		0	0							
	<i>B. paratyphosus</i> B BS† . . . . .		0	0							
	<i>B. aertrycke</i> L† . . . . .		0	0							
	<i>B. avisepticus</i> NYS 236† . . . . .		0	0		0	0				
	<i>B. welchii</i> H 2175 Bc (unheated) . . . . .	0	75	75	0	0	0			50	87
	<i>B. welchii</i> H 2175 Bc† . . . . .		56	53				100	94	99	94
	<i>B. welchii</i> H 2175 Bc (boiled $\frac{1}{2}$ hour) . . . . .		0	0						37	75
	<i>B. welchii</i> H 5932† . . . . .		0	0							
	<i>B. oedematiens</i> D† . . . . .		0	0							
III	<i>B. welchii</i> BP4 . . . . .		0	0							
	<i>Bact. monocytogenes</i> 53 xiii† . . . . .		0	0							
	<i>B. bovis-septicus</i> S . . . . .	0	0	0	0	0	0				
	<i>B. influenzae</i> B† . . . . .		0	0							
	<i>Strep. scarlatinae</i> † . . . . .		0	0							
IV	<i>B. typhosus</i> R† . . . . .		0	0							
	Human red cells I Jansky (fresh) . . . . .		0	0							
	Human red cells II Jansky (fresh) . . . . .		0	0							
	Human red cells III Jansky (fresh) . . . . .		0	0							
	Guinea pig red cells (fresh) . . . . .		0	0							
V	Rabbit red cells (fresh) . . . . .		0	0							
	Sheep muscle (fresh, Hb removed) . . . . .	0	0	0	0	0	0				
VI	Beef muscle (fresh, Hb removed) . . . . .		0	0							
	<i>Staph. albus</i> † . . . . .		0	0							
	Alpha hemolytic strep.† . . . . .		0	0							
	Gamma hemolytic strep.† . . . . .		0	0							
	Gamma hemolytic strep.† § . . . . .		0	0							
	<i>Staph. albus</i> (unheated)   . . . . .				0						
	Gamma hemolytic strep.   . . . . .				0						

\* These tissues were boiled for  $\frac{1}{2}$  hour on two successive days. Their heterophile antigen properties and content were intact.† Bacterial suspensions heated at 70° C. for  $\frac{1}{2}$  hour.

‡ Nose and throat cultures from Case 1.

§ Bacterium isolated from mouse injected with nose and throat swab washings of Case 1.

|| Throat cultures from Case 2.

appeared, so that the normal heterophile immune bodies had again become manifest.

These indications that antibodies of the type found in this disease are not a constituent of normal human serum suggest that they are a response to a specific etiological factor, either extrinsic or intrinsic, rather than the result of a non-specific stimulation as the consequence of an infectious or other process.

In the second group of tests absorption was carried out with all the tissues and practically all the bacteria known to contain the Forssman antigen. Of the tissues, most of which are known to be very polyvalent in their power to absorb heterologous heterophile antibodies, none removed the immune bodies for sheep corpuscles except horse kidney, which was quite irregular in even the moderate activity which it displayed. That horse kidney contains any component other than the heterophile antigen in common with sheep cells is very doubtful, in view of the fact that Bailey and Shorb (1933a) and Shorb and Bailey (1934) showed that the sheep cell hemolysin in anti-horse kidney rabbit serum is completely absorbed by various heterophile bacteria, and that similar tests which we have done failed to indicate such a relationship. It is seen that whereas the other antigens which successfully absorbed removed both agglutinins and lysins for sheep cells, horse kidney showed irregularity in this respect. The results obtained may be due, we believe, to excessive physical absorption by this tissue.

With the heterophile bacteria used in Group II of the tests, absorption of immune bodies was found only with one strain of *B. welchii* (H2175 Bc). The organisms which were completely negative have been extensively investigated as to their heterophile properties by Bailey and Shorb (1931, 1933a and b) and Shorb and Bailey (1934), who originally found the antigen in many of these bacteria. These authors state that "... some species, including *M. catarrhalis* (human), *B. avisepticus*, *Strep. viridans*, along with the *Diplo. pneumoniae* and *B. leipsepticus* heterophile cultures, have antigens with marked affinity for heterologous as well as homologous hemolysin. It is readily apparent that *B. leipsepticus* 370, *B. avisepticus* NYS 236, *Diplo. pneumoniae* DRI, *Strep. viridans* Cl, and to a less extent *M. catarr-*

*halis* E have a great affinity for the hemolysins produced in rabbits by most heterophile antigens whether bacterial or tissue. These results seem especially significant when it is considered that these organisms, in comparison with the other bacteria containing heterophile antigen, produce high hemolytic titers in rabbits when only very small amounts of the cultures are injected. These organisms apparently contain relatively large amounts of the antigen (taking into consideration the possible effect of chemical differences in antigens on heterologous absorption)." An example of this is afforded by the finding of these authors that each 0.1 to 0.2 cc. of sedimented bacteria (*Diplo. pneumoniae* DRI and *B. leipsepticus* 370) from 50 cc. of broth culture absorbed almost 2,000 units of hemolysin from Type I antipneumococcus rabbit serum. Each 10 units (0.1 to 0.2 cc.) of boiled sheep red cells removed 760 units of hemolysin from the same serum. It has been stated before that these investigators found normal human heterophile antibodies to be absorbed by these bacteria as well as the rabbit serum hemolysins.

The removal of antibodies by *B. welchii* H 2175 Bc is seen to be irregular in several respects. In the first place antibodies were not absorbed from the serum of Case 2 at all. With the serum of Case 1 the removal of antibodies for sheep cells was effected by unheated bacteria or organisms heated at 70° C. for 30 minutes, but not with bacteria which had been boiled for 15 minutes. In view of the heat stability of the antigen in ox and sheep erythrocytes this is not explicable, yet the tests were repeated several times with the same results. Furthermore, even the unheated bacteria did not absorb the ox cell lysins. However, in Case 3, the heated and unheated bacteria absorbed both sheep and ox antibodies. The results with this bacterium were so regular in the cases where absorptive activity was displayed (i.e., both agglutinins and hemolysins were removed) that it is difficult to consider the reactions other than as examples of specific absorption. Another strain of the same bacterium (H 5932) which also has been shown by Shorb and Bailey to contain the Forssman antigen, did not absorb the antibodies.

No simple explanation is available to us for the irregularities of absorption by *B. welchii* H 2175



Bc which were observed. But if despite this we assume that this antigen under certain conditions is capable of specific absorption of the infectious mononucleosis antibodies, the comments of Shorb and Bailey on the nature and properties of the heterophile antigen contained in this particular strain of *B. welchii* are of much interest. These authors state: "It can be concluded . . . that the heterophile antigens contained in some species of bacteria, especially that of . . . *B. oedematiens*, *B. welchii* as well as *B. paratyphosus* B and *B. dysenteriae* Shiga are quite specific since even the homologous strain or other strains of the same species may not absorb completely the species hemolysin. *B. welchii* antiserum (H 2175 Bc) is of interest because with certain samples very little absorption was noted with any of the bacteria except the homologous antigen. In some cases the hemolysin was not even absorbed by any of the heterophile tissue antigens which are known to have a great affinity for heterologous heterophile hemolysin. The significance of the lack of combining ability is not known. It may be that this particular organism, besides producing heterophile hemolysin, produces an hemolysin of another type not entirely related to the receptors found in sheep cells or guinea pig tissues."

Agglutination tests were done with the serum of Case 1 and the three strains of *B. welchii* used in the absorption tests (H 2175 Bc and H 5932 are supposed heterophile strains, while BP4 is non-heterophile). The tests were not conclusive. There was settling out of the H 2175 Bc strain in tubes containing serum in dilution up to 1 to 10,240, but no settling in control tubes containing normal human serum and saline. With the other two strains there was no settling in any tubes. This may be of some significance, for the fact that no clumping was seen may be due to the difficulty encountered in agglutination of encapsulated bacteria.

Group III of the tests includes a variety of non-heterophile bacteria which were tested more or less at random, though with regard to the symptoms of the diseases with which they are associated, with the object in mind that a possible etiological relationship to the disease might be encountered. Paul and Bunnell and Bernstein have already ascertained that patients with the diseases caused by most of these organisms (influenza, scarlet fever, typhoid fever) do not have

increased titers for sheep cells. *B. bovissepticus* was tested because we found, in another connection, that it seems to have a peculiar agglutinogenic relationship to sheep erythrocytes. We were particularly interested in the test with *Bact. monocytogenes*. Murray, Webb and Swann (1926) discovered this organism as the etiological basis for a disease of rabbits characterized by a pronounced monocytosis. Dr. Murray kindly sent us this organism. The absorption tests which we carried out with it were negative. We were also interested in the apparently related organism, *Bact. monocytogenes hominis* of Nyfeldt (1929). Unfortunately, absorption tests with this bacterium were not reported by the author since the hemolytic antibodies associated with the disease were unknown at the time his work was done. If such an organism could be shown to absorb the specific type of ox and sheep cell antibodies discussed in this paper, a rather firm basis for its etiologic relationship to infectious mononucleosis would be established. We are attempting to carry out this phase of the problem at the present time.

The fourth group of tests was carried out with various erythrocytes, none of which absorbed. Of particular interest here is the human Group A (Jansky Group II) corpuscles. It is generally accepted that the Forssman antigen and human Group A agglutinogen are similar but not identical. Bernstein discusses the possibility that in infectious mononucleosis red cells are broken down with liberation of the antigen which subsequently produces the antibodies. He states further that there is ample evidence that such is not the case, since infectious mononucleosis and the sheep cell antibodies appeared in patients of other blood groupings with as great regularity as in Groups A or AB, nor did several patients receiving multiple transfusions with Group A blood develop an increased antibody titer. The tests reported here further minimize the possibility of normal red cells acting as an intrinsic stimulation for the antibodies found in this disease.

The remainder of the tests demonstrate that the antigen is not present in fresh beef or sheep muscle washed free of hemoglobin, and that nose and throat cultures from two of the patients failed to absorb hemolysins or hemagglutinins from the sera of these persons.



*Other tests for Forssman antibody.* There are several methods for the demonstration of heterophile antibody besides those already discussed. Thus, both human and rabbit heterophile sera when introduced intravenously into guinea pigs are known to produce generally a syndrome very similar to anaphylactic shock, while intracutaneous injection into these animals causes a severe local necrotic reaction. This property of the serum is known as its primary toxicity. The work of a number of investigators has shown, however, that such primary toxicity does not parallel the heterophile antibody content of the sera in all instances, especially in the case of certain of the antibacterial heterophile sera. With these facts in mind we have tested the serum from a case of infectious mononucleosis by injection into the guinea pig. An intracutaneous test done with 0.2 cc. of serum from Case 2 with an agglutinin titer of 350 for sheep cells was entirely negative. We have not had an opportunity to carry out further tests with sera of higher titers for sheep cells.

Among the characteristic reactions manifested by heterophile hemolytic antibodies are (1) combination with emulsions of lipoids derived from heterophile tissues, and (2) formation of precipitates with emulsions of such lipoids. The heterophile lipoid is known to be soluble in alcohol. Such lipoid preparations, which have been found effective when used with known heterophile antibodies, were tested with the infectious mononucleosis sera for the above properties and found negative. Further studies with other solvents are now being made.

*The value of ox cell absorption in the diagnosis of infectious mononucleosis.* Abnormal titers of antibodies for sheep erythrocytes have been found consistently in human beings only in two conditions; following horse serum therapy and in infectious mononucleosis. Aside from these cases high concentrations of these immune bodies have been reported in isolated instances only. Ramsdell and Davidsohn (1930) reported a titer above the normal in a patient receiving injections of insulin. Paul and Bunnell discuss a fatal case in which the serological findings were those of infectious mononucleosis, but the clinical manifestations were too indefinite to permit a satisfactory diagnosis, so that it was classified as either aleukemic leukemia or aplastic anemia.

Despite the fact that the combination of diagnostic factors in infectious mononucleosis is such that the correct diagnosis would seem a comparatively simple matter, this is apparently not always the case. Bernstein, for example, reports a case in which the syndrome was that of thrombocytopenic purpura, accompanied by a high sheep cell agglutinin titer, and considers the possibility of a concurrent atypical infectious mononucleosis. Rosenthal and Wenkebach (1933) discuss the relationship of this disease to the group of conditions which they consider similar but distinct entities. This group includes lymphocytic angina, monocytic angina and glandular fever. These authors state that despite the lack of certain symptoms the diagnosis may be easily confirmed by the positive heterophile antibody reaction. In some of these cases agglutinin titers of 16 (which are sometimes found in normal individuals and after horse serum treatment) serve as the basis for including the disease under the classification of infectious mononucleosis.

In instances where reliance is placed chiefly on the sheep cell hemagglutinin titer for the diagnosis of this condition, two main sources of error are to be considered. In the first place Bernstein has pointed out that there is a wide range of antibody concentration in normal people, and "that if the increased titer of agglutinins in infectious mononucleosis merely represents an enhancement of the concentration of antibodies already present, then there is just as much increase in a patient's serum with a normal agglutinin titer of 1 to 1, rising to 1 to 16 as there would be in one starting at 1 to 8 and rising to 1 to 128. Yet the former would be considered a negative heterophile antibody test and the latter a positive one." Secondly, the possibility of a high heterophile titer due to previous horse serum injections is a factor of some importance. Davidsohn (1929) has pointed out that sheep cell agglutinins may persist for long periods after such treatment. In one patient these were present one year after injection of serum. In another who had received diphtheria antitoxin three years before, and in two similarly treated two years before, these immune bodies were still present. Bernstein emphasizes the importance of eliminating horse serum as an inciting agent before drawing any conclusions from an increased titer of heterophile antibodies.

In the normal individuals whom we have tested, we have been able to find no antibodies of the nature of those found in infectious mononucleosis. Likewise, the cases of this disease which we have studied have shown the disappearance of these and the presence of the true heterophile antibodies after recovery, as discussed in a preceding section. From the fact also that previous investigators (Paul and Bunnell, Boveri, Bernstein) have shown that none of a large variety of pathologic conditions tested were characterized by the presence in the blood of sheep cell antibodies, and since such antibodies in serum sickness are actually heterophile in nature, we believe that the antibody seen in infectious mononucleosis is specifically characteristic of this disease. If it can be demonstrated that one immune body for both sheep and ox cells is present in measurable concentration in an individual suspected of harboring the infection, then so far as is known there is no serological basis to hinder classification of the condition as infectious mononucleosis. The logical manner in which such a test should be carried out is the primary titration of the serum with sheep cells and then absorption of the serum with boiled or autoclaved ox cells, followed by titration against sheep corpuscles. This method is advised because it provides immediate proof that one antibody is responsible for the activity of the serum against both species of erythrocytes. Furthermore, in addition to the commonly used sheep cells this method involves the use only of boiled ox cells, which can be kept sterile in the icebox almost indefinitely for use at any time.

The value of this type of test in certain vague conditions was especially impressed on us by a case in which we were interested as a possible example of infectious mononucleosis. A detailed report follows:

**Case 4.**<sup>7</sup> H. M., an 8 year old white male, was admitted to the Jewish Hospital of Brooklyn on March 11, 1934, complaining chiefly of a fever of 10 days duration. The history indicated that the patient had suffered with diphtheria some time in the past.

On admission the child did not seem acutely ill. The skin was faintly tinged a lemon yellow and the pharynx

was moderately injected. There was no adenopathy and the splenic margin was questionably palpable. During the past 10 days the temperature had been remittent up to 102° F., though on two occasions it had risen to 105° and 105°. There were no other complaints.

Through the first six days of hospitalization the temperature showed a daily intermittent course up to 103° in the evening, being normal in the morning. There were still no other complaints. Nose and throat cultures as well as blood and spinal fluid cultures were negative during this interval. The Widal was negative on the day of admission and agglutination tests a week later with the *B. melitensis* and *abortus* group indicated no abnormality.

At the time of admission the total leukocyte count was 10,000 and the differential picture showed a monocytosis. Subsequent blood studies are illustrated in Figure 4. Of

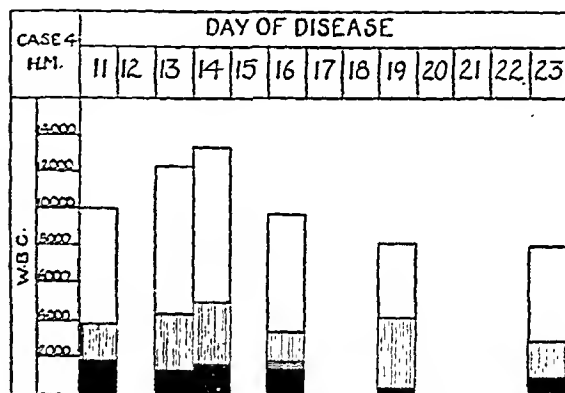


FIG. 4. A SUSPECTED CASE OF INFECTIOUS MONONUCLEOSIS IN WHICH ANTIBODIES FOR OX ERYTHROCYTES WERE LACKING.

Legends as in Figure 1.

particular interest is the fact that two days later it was observed that 1 or 2 young lymphocytes were present, but the change in these cells was not striking. Three days after this, on March 16, the notation was made that 4 lymphocytes typical of the cells seen in infectious mononucleosis were found, though at this time the total lymphocyte percentage was only 21. The degenerative index of polymorphonuclear cells was 64 per cent, indicating a toxic blood picture. The impression recorded in the history on this date was that of infectious mononucleosis. The next day agglutinins for sheep cells were found in 1 to 32 dilution of this patient's serum.

Several days later no abnormal lymphocytes were found, so that the possibility of infectious mononucleosis was excluded.

The patient continued to run a septic temperature daily with no chills and no other complaints. On April 7, 1934, he was discharged from the hospital with the fever undiagnosed.

<sup>7</sup> The authors are indebted to Dr. M. R. Greene of the Jewish Hospital of Brooklyn, New York, for supplying clinical data and serum in this case.

A sample of serum from this patient was obtained on March 31, thirty days after the beginning of the illness. At this time the titer for sheep cells was quite high, complete lysis being found in dilution of 1 to 160. The agglutinins were not parallel, being definitely positive through 1 to 16 dilution. Hemolysins for ox cells were of a low normal value. Absorption tests with this serum showed that ox cells did not remove the sheep cell antibodies, whereas carp gill and guinea pig kidney did. The antibodies were therefore heterophile and as such not compatible with the findings made in infectious mononucleosis. It was mentioned in the case report that this child had a history of diphtheria. We were unable to ascertain how long before the present illness he had suffered with this disease nor whether antitoxin had been administered. There is obviously a possibility that the sheep cell antibodies found might have had their origin from the treatment of the earlier illness.

#### DISCUSSION AND SUMMARY

Previous investigators who have reported on the antibody responses in infectious mononucleosis have used the terms heterophile or Forssman antibody in describing the sheep red cells agglutinin and hemolysin first observed by Paul and Bunnell in this disease. It has been assumed that these immune bodies are true Forssman antibodies because of their action on sheep cells, although no tests with other heterophile or non-heterophile antigens are recorded.

The complex relationships between the antigenic components of various species of red blood cells and tissues and the corresponding antibodies have led to a confusing terminology in their description, but the essential points concerning these relationships are quite simple and are well known. The antigenic substances that incite the production of hemolysins in the rabbit for sheep erythrocytes have been divided into two classes, heterophile and isophile. The heterophile antigens most commonly mentioned include the red cells of the sheep and goat, the organs of the guinea pig, horse, cat, dog, mouse, chicken, tortoise, certain kinds of fish and several varieties of bacteria. The common non-heterophile antigens include the organs of the rabbit, ox, pig, man, rat, goose, pigeon, frog, etc. Animals whose organs or red

cells contain heterophile antigen are sometimes classed as of the "guinea pig type"; those which do not, the "rabbit type." The red cell of the ox is generally mentioned as an example of a typical isophile antigen, while the phylogenetically closely related sheep red cell contains both heterophile and isophile components. Both of these types of antigens can be recognized and defined in terms of the behavior of their antibodies. It has generally been stated that a true heterophile hemolytic antibody is completely removed from its serum by boiled sheep red corpuscles (and heterophile tissues, such as guinea pig kidney, etc.), while the isophile hemolysin is absorbable only by unheated sheep or ox corpuscles. The hemolysins produced in the rabbit by the injection of native sheep corpuscles possess both heterophile and isophile properties. Sheep corpuscles therefore contain at least two kinds of lysinogens,—a heterophile fraction which is thermostable and an isophile component which is thermolabile. This fact is demonstrated not only by the reactions of anti-sheep hemolysins toward native and boiled sheep cells, but also by the fact that only heterophile immune body results when the boiled cells are injected into rabbits.

The red cells of the ox incite in the rabbit hemolytic antibodies for the red cells of the sheep as well as for the homologous cells. The ox cell is of isophile type, since its antibody combines only with the thermolabile receptors of sheep and ox corpuscles. Hyde (1925) has shown that this anti-ox hemolysin is removed from its serum by native, but not by boiled corpuscles of these two species.

The results of previous investigations have shown then that sheep and ox erythrocytes are related antigenically through their thermolabile components, while the thermostable components of these two species of cells have not been shown to have anything in common. The results of our own study with these erythrocytes demonstrate a relationship between certain thermostable fractions of these cells as indicated by the ability of autoclaved ox and sheep erythrocytes mutually to cross absorb the hemolytic and hemagglutinative antibodies for both of these species of cells from the serum of patients with infectious mononucleosis. Our results demonstrate further that the

antibodies in infectious mononucleosis are not of the heterophile (Forssman) type. They are probably the response to an antigen which is similar to a hitherto unknown heat stable component of the ox and sheep erythrocyte, and also of a bacterium, *B. welchii* H 2175 Bc, and possibly of horse kidney. Immune bodies of this type apparently are not a constituent of normal human blood, nor are they found in any of a large variety of pathological conditions as indicated by an analysis of the data of previous investigators who have been concerned with the heterophile antibody content of such sera. They are therefore limited strictly to this disease and for this reason may serve as the basis for a diagnostic test, particularly when other features of the syndrome cannot be distinguished with certainty, and even though the history indicates previous horse serum therapy. Cases have been reported (Bernstein) wherein the clinical findings were those of infectious mononucleosis but appreciable agglutinins for sheep erythrocytes were lacking. Whether such instances would show high titers for ox cells we have had no opportunity to determine, but obviously observations on this point would be of much interest. We believe that even when high titers for ox erythrocytes cannot be found, the demonstration that such low concentration of antibody as is present is of the type reported in this paper would serve to class the disease as infectious mononucleosis.

The etiology of this condition is still a matter of conjecture. The peculiar properties of the antibody concerned gives us, however, a more definite basis than was heretofore available for testing the possible relationship of various bacteria or other factors to the disease. Since the antibody is characteristic of this condition and is not found in normal serum, we believe that it is a specific response to a definite factor, either intrinsic or extrinsic, rather than a reaction due to non-specific stimulation. The recognition of an extrinsic factor which would combine with this immune body should logically supply a strong indication that it plays a specific part. On the other hand, we may conclude from the fact that nose and throat organisms from our own cases failed to absorb these antibodies, that if the responsible causative agent is found in these regions, it is not culturable on ordinary media.

In this connection it is interesting that abnormal concentrations of bacterial agglutinins are present in some cases of this disease. Bernstein has reported instances in which agglutinins for the typhoid and suipestifer groups of organisms were found. Case 1 reported here was one of the patients showing such antibodies for the typhoid group, while Case 2 agglutinated *B. melitensis* to an abnormal degree. Some of these bacteria have been tested for their ability to absorb the mononucleosis antibodies, and have been found negative. In view of this, and also because the antibacterial response is seen in only few cases, and not to the same bacteria in these instances, we are inclined to favor the opinion held by Bernstein that such agglutinins are not specifically related to the disease. They are probably the result of non-specific stimulation.

A consideration of the serological findings in this condition brings up several points of theoretical interest. The sheep cell agglutinin titers parallel those for hemolysins. With the exception of the isoagglutinins of the human blood groups and the autohemagglutinins in rabbits following repeated blood transfusions, we know of no instance either in human beings or experimental animals where the agglutinins for erythrocytes of any kind are as high as the hemolysins. In most cases the former do not even approach the latter. Our knowledge of the properties of human anti-erythrocyte sera is necessarily very limited, of course. It may possibly be that investigation of the responses of the immunized human being to various corpuscles would disclose a natural tendency of this species to produce higher agglutinin titers than are found in experimental animals.

The controversial ideas concerning the essential identity of antibodies, generally termed the unitarian hypothesis, are too well known to warrant discussion here. We merely wish to comment on the possible bearing of certain results reported in this communication on this theory. It was seen that ox erythrocytes remove both agglutinins and hemolysins for sheep corpuscles. It seems very improbable that the ox cell would contain two factors, one with an affinity for the agglutinin and the other with an affinity for the hemolysin. It seems to us rather more probable that one antigenic component is responsible for the absorption of both immune body manifestations.

## CONCLUSIONS

1. The hemolytic and hemagglutinative antibodies for sheep and ox red cells found in the sera of cases of infectious mononucleosis are not heterophile or Forssman antibodies, but are probably the specific response to an antigen having a factor in common with a thermostable component of sheep and ox erythrocytes, a certain strain of *B. welchii* (H 2175 Bc), and possibly horse kidney.

2. These antibodies have the power to hemolyze and agglutinate not only sheep red cells, but also to hemolyze and to some extent agglutinate ox red corpuscles, although these immune bodies are not isophile in nature since they are absorbed from the serum by boiled ox and sheep corpuscles.

3. Ox erythrocytes have a broader antigenic relationship to the antibodies of infectious mononucleosis than sheep erythrocytes.

4. The antibodies are probably not found in normal human serum, but are rather a specific response associated with infectious mononucleosis only.

5. These antibodies were not absorbed from the serum by any of the organisms cultivated from the upper respiratory tract of cases of the disease.

6. Blood cultures made from cases of infectious mononucleosis were negative, although the injection into rabbits of such cultures, containing the blood elements of a patient, caused the production of a serum with a high hemolytic titer for sheep cells.

7. The antibodies in infectious mononucleosis and the thermostable antigen in ox and sheep cells which combine with and neutralize them have unique properties of specificity which so far as we are aware have not been described before.

8. The primary titration of a serum with fresh sheep erythrocytes followed by absorption of the antibodies with boiled ox red cells and then re-titration of the absorbed serum with fresh sheep corpuscles, provides a specific diagnostic method for this disease.

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# STUDIES IN SERUM ELECTROLYTES. IX. THE CHANGE IN TOTAL QUANTITY AND OSMOLAL CONCENTRATION OF GLUCOSE AND CHLORIDE IN THE SERUM AFTER THE INGESTION OF GLUCOSE BY DIABETIC PATIENTS

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Under certain conditions observers have reported an inverse relationship in the concentration of sugar and of chloride in the blood. Herrick (1) in 1924 observed it following the ingestion of 100 grams of glucose in six individuals. Ni (2) obtained the same relationship following the removal of the pancreas or the administration of insulin in dogs which he regarded as a manifestation of osmotic compensation. Sunderman, Austin and Williams (3) observed it following the administration of insulin to diabetic patients,—the increase in serum chloride being associated with an increase of total fixed base. Their freezing point data suggested that the increased concentration of electrolytes in the serum did not entirely compensate osmotically for the decreased concentration of sugar. They found the change usually accompanied by a higher specific gravity and an increased concentration of the total solids of the serum.

Changes in the concentration of blood sugar are usually associated with changes in the water content of the blood and tissues. The intravenous administration of hypertonic solutions of glucose is used clinically to produce dehydration and diuresis. Following the administration of hypertonic solutions of either saccharose or glucose intravenously in dogs, Keith (4) found the plasma volume diminished and both the viscosity of the blood and the concentration of hemoglobin increased. Drabkin, Page and Edwards (5) and Drabkin (6) demonstrated that administration of insulin to dogs produces not only a lowering of the blood sugar but also anhydremia which manifests itself in increase of the concentration of hemoglobin and erythrocyte count, and reduction of plasma volume.

Change in the concentration of a solute may re-

sult from addition or removal of either solute or solvent. Moreover, in solutions such as blood or serum which contain a high concentration of solids, the distinction between concentration of solute per unit of solution and per unit of solvent is important. The concentration of solute is commonly estimated in relation to volume of solution in clinical studies. It is, however, the concentration of solute per unit of solvent which perhaps has more physicochemical significance.

The present study was designed to determine in the diabetic patient to whom glucose was administered the *changes* in the concentration of glucose and chloride in serum in relation to the *change* in the amount of water circulating in the serum, and from these data to estimate the *change* in the total quantity and in the osmolal concentration of both glucose and chloride in serum.

## MATERIAL AND METHODS

Venous blood was withdrawn under oil from 18 fasting patients who were attending the Metabolic Clinic at the Pennsylvania Hospital for diabetes mellitus. The serum was obtained from the centrifuged specimen of the clotted blood by the technique described elsewhere (7). Seventy-five grams of glucose dissolved in 200 cc. of water flavored with lemon juice were then ingested, and after an interval of ninety minutes serum was again obtained for analysis.

Glucose in the serum was measured by Benedict's method (8) within 20 minutes after the collection of the blood. The Wilson and Ball procedure (9) was employed for the measurement of the serum chloride. The specific gravity of the serum was measured at 20° C. with pycnometers of 2 ml. capacity. The total residue of the serum was obtained by drying the serum at 100° C. to constant weight. Freezing point measure-

<sup>1</sup> Resident in Chemistry, Pennsylvania Hospital.



TABLE I  
Effect of ingestion of glucose by diabetic and normal individuals on the glucose and chloride concentrations of serum

Case	Specific gravity	Chloride <i>m. Eq./L</i>	Glucose <i>mM./L</i>	Solids <i>grams/kgm.</i>	Chloride <i>m. Eq./kgm. H<sub>2</sub>O</i>	Glucose <i>mM./kgm. H<sub>2</sub>O</i>	$\Delta$ [Glucose] - $\Delta$ [Chloride]	Chloride <i>m. Eq./kgm. R</i>	Glucose <i>mM./kgm. R</i>	Water <i>grams/kgm. R</i>	Solution transferred to serum after glucose					$\tau_{\text{ex}}$ <i>mm.</i>
											Chloride <i>m. Eq./kgm. R</i>	Glucose <i>mM./kgm. R</i>	Water <i>grams/kgm. R</i>	Chloride <i>m. Eq./kgm. H<sub>2</sub>O transferred</i>	Glucose <i>mM./kgm. H<sub>2</sub>O transferred</i>	
1 (f).....	1.0290	103.0	8.9	91.55	110.2	9.5	4.77	1189.5	102.7	10796	39.1	122	576	67.9	211.8	347.6
2 (f).....	1.0285	101.2	18.5	89.10	108.0	19.8		1228.6	224.7	11372						
3 (f).....	1.0281	97.8	16.4	88.00	104.3	17.5	3.99	1195.3	200.9	11463	101.2	182	1353	74.8	134.5	284.1
4 (f).....	1.0269	99.9	28.2	82.00	101.2	29.9		1296.5	382.9	12816						
5 (f).....	1.0276	99.9	17.4	90.25	106.8	18.6	4.46	1192.0	207.8	11160	33.5	210	727	46.0	288.9	380.9
6 (f).....	1.0273	96.6	32.9	88.00	103.1	35.2		1225.5	417.8	11887						
7 (f).....	1.0274	102.4	9.3	92.00	109.7	9.9	3.73	1178.7	106.9	10740	94.2	161.1	1223	77.0	131.7	285.7
8 (f).....	1.0268	100.9	21.0	85.80	106.4	22.4		1272.9	268.0	11963						
9 (f).....	1.0304	100.9	12.7	95.90	108.3	13.7	3.11	1113.4	140.3	10280	-1.3	137.2	378	-3.4	363.0	356.2
10 (f).....	1.0302	97.3	24.3	94.70	104.3	26.0		1112.1	277.5	10658						
11 (f).....	1.0291	100.1	8.5	93.95	107.3	9.1	4.51	1120.8	94.7	10442	103.6	147.7	1247	83.1	118.4	284.6
12 (f).....	1.0268	98.2	19.4	87.10	104.8	20.7		1224.4	242.4	11689						
13 (f).....	1.0255	100.4	7.8	88.80	107.2	8.3	3.89	1195.7	92.5	11150	76.1	215.5	1203	63.3	179.1	305.7
14 (f).....	1.0253	100.4	23.4	83.80	103.0	24.9		1271.8	308.0	12353						
15 (f).....	1.0286	108.6	8.7	80.65	115.2	9.2	3.34	1451.6	115.5	12600	5.4	109.2	328	16.5	332.9	365.9
16 (f).....	1.0285	101.9	16.7	86.90	106.4	17.8		1457.0	224.7	12928						
17 (f).....	1.0275	106.3	11.2	86.25	113.2	11.9	4.40	1209.0	197.0	11096	61.8	151.4	852	72.5	177.7	322.7
18 (f).....	1.0271	103.9	23.2	82.90	110.3	24.7		1322.4	310.4	12577						
19 (f).....	1.0258	103.9	12.4	80.55	110.2	13.2	4.34	1398.2	167.1	12691						
20 (f).....	1.0260	101.1	22.5	79.35	107.0	23.8		1387.3	314.7	13217	15.8	147.6	526	30.0	280.6	340.6
21 (f).....	1.0308	102.8	13.3	92.40	110.6	14.3	3.49	1107.9	143.2	10015	73.6	123.2	949	77.6	129.8	285.0
22 (f).....	1.0290	100.6	22.7	90.25	107.8	24.3		1181.5	266.4	10964						
23 (f).....	1.0268	98.5	11.5	88.45	105.2	12.3	2.35	1186.3	138.7	11278	78.1	65.0	960	81.4	67.7	230.5
24 (f).....	1.0255	97.2	15.7	83.20	103.3	16.7		1264.5	203.7	12238						
25 (f).....	1.0254	98.2	12.9	78.90	104.0	13.7	3.45	1348.1	177.3	12965	11.5	204.5	655	17.6	312.2	347.4
26 (f).....	1.0257	94.4	26.5	77.75	99.8	28.0		1359.6	381.8	13620						
27 (f).....	1.0295	105.3	7.7	94.20	112.9	8.3	2.95	1177.4	86.2	10426	80.2	88.7	955	84.	92.9	260.9
28 (f).....	1.0286	103.6	14.4	88.50	110.5	15.4		1257.6	174.9	11381						
29 (f).....	1.0268	104.8	6.0	85.55	111.6	6.4	3.83	1209.6	74.5	11644	65.9	128.4	878	75.1	146.2	296.4
30 (f).....	1.0258	102.7	15.3	81.85	109.1	16.2		1365.5	202.9	12522						
31 (f).....	1.0263	102.1	9.8	84.50	108.6	10.4	4.38	1292.1	123.8	11894	88.1	147.6	1096	80.4	134.7	295.5
32 (f).....	1.0261	100.3	19.7	80.00	106.3	20.9		1380.2	271.4	12990						
33 (f).....	1.0258	105.4	10.8	84.20	112.2	11.5	3.44	1346.5	137.6	12002	98.2	128.7	1173	83.7	109.7	276.8
34 (f).....	1.0250	103.5	20.0	79.15	109.7	20.2		1444.7	266.3	13175						
Normal																
FVS (f).....	1.0269	101.3	5.7	89.00	108.3	6.1	$\infty$	1200.2	67.1	11084						
ESV (f).....	1.0268	101.3	4.7	89.15	108.3	5.0		1194.9	52.2	11032	5.3	12.0	52	102.1	231.5	435.1
ESV (f).....	1.0270	102.2	5.1	85.05	108.8	5.5	3.43	1270.4	64.0	11680	2.8	28.1	109	25.8	259.4	311.0
ESV (f).....	1.0271	102.9	2.9	85.40	109.5	3.1		1267.6	35.9	11572						

ments were made by means of the Stadie-Sunderman apparatus (10).

#### CALCULATION OF RESULTS

The concentrations of glucose and chloride in serum before and after the glucose administration have been expressed as moles per liter of serum; as moles per kilogram of serum water; and as moles per kilogram of dry residue exclusive of the glucose and chloride. It is the second of these, the molality of a solute in serum which is most simply related to changes in the osmotic equilibrium, thermodynamic potential, or activity of the solute. If it be assumed as a plausible approximation for these experiments, that, excluding the glucose and the chlorides, the remaining solids of the serum remain constant during the ninety minute period of the experiment, then from the ratios of glucose, chloride, and water to solids the percentile change in the total quantities of these three components may be calculated.

The following equations were employed in our calculations (those for glucose being analogous to those for chloride).

#### SYMBOLS

- Sp = specific gravity at 20° C.  
 H<sub>2</sub>O = water in grams  
 S = solids in grams  
 Gl = glucose in millimoles  
 Cl = chloride in millimoles  
 L = liters of serum  
 K = kilograms of serum  
 [ ] = concentration per kilogram of water  
 R = residual solids in kilograms. (Solids exclusive of glucose and chloride as NaCl)  
 $\pi_{ex}$  = osmolal concentration of glucose and chloride in the fluid calculated as exchanged  
 subscript f = fasting  
 subscript g = after glucose

$$\frac{Cl/L}{Sp} = Cl/K; \quad \frac{H_2O/L}{Sp} = H_2O/K = 1000 - S/K, \quad (1)$$

$$\frac{1000 Cl/K}{1000 - S/K} = [Cl], \quad (2)$$

$$Cl/R = \frac{1000 Cl/L}{Sp (S/K) - 180 Gl/L - 58.45 Cl/L}, \quad (3a)$$

$$H_2O/R = \frac{Sp \times 10^5 - Sp (S/K) \times 10^3}{Sp (S/K) - 180 Gl/L - 58.45 Cl/L}. \quad (3b)$$

Transference of H<sub>2</sub>O, Cl, and Gl to the serum after glucose administration.

$$\Delta Cl/R = (Cl/R)_g - (Cl/R)_f, \quad (4a)$$

$$\Delta H_2O/R = (H_2O/R)_g - (H_2O/R)_f. \quad (4b)$$

Concentrations of Cl and Gl (expressed as mM. per kilogram of water added) in the solution entering serum after glucose administration are as follows:

$$\Delta Cl/(\Delta H_2O \times 10^{-3}) = \frac{1000 \Delta Cl/R}{\Delta H_2O/R}. \quad (5)$$

Estimated osmolal concentration of chloride and glucose in the fluid calculated as exchanged ( $\pi_{ex}$ ) assuming 1.9 as the osmolal equivalence of a mole of electrolyte.

$$\pi_{ex} = 1.9 \Delta Cl/(\Delta H_2O \times 10^{-3}) + \Delta Gl/(\Delta H_2O \times 10^{-3}). \quad (6)$$

#### RESULTS

The data pertinent to this paper have been recorded in Table I.

#### *Relationship between the concentrations of glucose and chloride in serum on a water basis*

From calculations made from the data in this paper and in a previous paper (11) there was no significant correlation between the concentration of glucose and chloride in either the serum or corpuscles in the blood of different patients. However, following the ingestion of glucose in diabetic patients there was a striking correlation between the change in the concentrations of glucose and chloride in the serum when these were expressed on a water basis.

In the serum of each of the diabetic patients following the administration of glucose there was a decreased concentration of chloride as well as the anticipated increased concentration of glucose. The increase in the glucose concentration in moles per kilogram of water was accompanied by a decrease in the chloride concentration in moles per kilogram of water in the mean ratio of 3.77  $\pm$  0.45 to 1.

In Figure 1 the increase in the milliosmolal concentration of glucose in the serum is plotted against the decrease in the milliosmolal concentration of chloride. The statistically calculated regression line of chloride on glucose is:

$$-\Delta \pi_{Cl} = 0.369 \Delta \pi_{glucose} + 1.50.$$

If the osmolal decrease in concentration of chloride were equal to the osmolal increase in glucose the curve would follow the dotted line. The increase in the concentration of glucose when greater than about 3 mM. per kilogram of water is thus not entirely compensated for osmotically by the decrease in the concentration of chloride.



TABLE I  
Effect of ingestion of glucose by diabetic and normal individuals on the glucose and chloride concentrations of serum

Case	Specific gravity	Chloride	Glucose	Solids	Chloride	Glucose	$\Delta$ [Glucose] - $\Delta$ [Chloride]	Chloride	Glucose	Water	Solution transferred to serum after glucose					
											Chloride	Glucose	Water	Chloride	Glucose	$\pi_{ex}$
1 (f) (g)	20/20° 1.0290 1.0283	m. Eq./L 103.0 101.2	mM./L 8.9 18.5	grams/kgm. 91.55 89.10	m. Eq./kgm. H <sub>2</sub> O 110.2 108.0	mM./kgm. H <sub>2</sub> O 9.5 19.8	4.77	m. Eq./kgm. R 1189.5 1228.6	mM./kgm. R 102.7 224.7	grams/kgm. R 10796 11372	m. Eq./kgm. R 39.1	mM./kgm. R 122	grams/kgm. R 576	m. Eq./kgm. H <sub>2</sub> O transferred 67.9	mM./kgm. H <sub>2</sub> O transferred 211.8	$\pi_{ex}$ mm. 347.6
2 (f) (g)	1.0285 1.0268	97.8 95.4	16.4 28.2	88.00 82.00	104.3 101.2	17.5 29.9	3.99	1195.3 1296.5	200.9 382.9	11463 12816	101.2	182	1353	74.8	134.5	284.1
3 (f) (g)	1.0281 1.0273	99.9 96.6	17.4 32.9	90.25 88.00	106.8 103.1	18.6 35.2	4.46	1192.0 1225.5	207.8 417.8	11160 11887	33.5	210	727	46.0	288.9	380.9
4 (f) (g)	1.0276 1.0269	102.4 99.9	9.3 21.0	92.00 85.80	109.7 106.4	9.9 22.4	3.73	1178.7 1272.9	106.9 268.0	10740 11963	94.2	161.1	1223	77.0	131.7	285.7
5 (f) (g)	1.0304 1.0302	100.9 97.3	12.7 24.3	95.90 94.70	108.3 104.3	13.7 26.0	3.11	1113.4 1112.1	140.3 277.5	10280 10658	-1.3	137.2	378	-3.4	363.0	356.2
6 (f) (g)	1.0291 1.0268	100.1 98.2	8.5 19.4	93.95 87.10	107.3 104.8	9.1 20.7	4.51	1120.8 1224.4	94.7 242.4	10442 11689	103.6	147.7	1247	83.1	118.4	284.6
7 (f) (g)	1.0274 1.0262	100.4 96.8	7.8 23.4	88.80 83.80	107.2 103.0	8.3 24.9	3.89	1195.7 1271.8	92.5 308.0	11150 12353	76.1	215.5	1203	63.3	179.1	305.7
8 (f) (g)	1.0255 1.0253	108.6 106.3	8.7 16.4	80.65 80.10	115.2 112.7	9.2 17.4	3.34	1451.6 1457.0	115.5 224.7	12600 12928	5.4	109.2	328	16.5	332.9	365.9
9 (f) (g)	1.0286 1.0285	101.9 99.9	16.7 27.4	90.65 86.90	109.0 106.4	17.8 29.2	4.40	1209.0 1270.8	197.0 348.4	11096 11948	61.8	151.4	852	72.5	177.7	322.7
10 (f) (g)	1.0275 1.0271	106.3 103.9	11.2 23.2	86.25 82.90	113.2 110.3	11.9 24.7	4.34	1322.4 1387.3	138.9 310.4	11678 12577	64.9	171.5	899	72.2	190.8	335.2
11 (f) (g)	1.0258 1.0260	103.9 101.1	12.4 22.5	80.55 79.35	110.2 107.0	13.2 23.8	3.35	1398.2 1414.0	167.1 314.7	12691 13217	15.8	147.6	526	30.0	280.6	340.6
12 (f) (g)	1.0308 1.0290	102.8 100.6	13.3 22.7	98.20 92.45	110.6 107.8	14.3 24.3	3.49	1107.9 1181.5	143.2 266.4	10015 10964	73.6	123.2	949	77.6	129.8	285.0
13 (f) (g)	1.0268 1.0255	98.5 97.2	11.5 15.7	88.45 83.20	105.2 103.3	12.3 16.7	2.35	1186.3 1264.5	138.7 203.7	11278 12238	78.1	65.0	960	81.4	67.7	230.5
14 (f) (g)	1.0254 1.0257	98.2 94.4	12.9 26.5	78.90 77.75	104.0 99.8	13.7 28.0	3.45	1348.1 1359.6	177.3 381.8	12965 13620	11.5	204.5	655	17.6	312.2	347.4
15 (f) (g)	1.0295 1.0286	105.3 103.6	7.7 14.4	94.20 88.50	112.9 110.5	8.3 15.4	2.95	1177.4 1257.6	86.2 174.9	10426 11381	80.2	88.7	955	84.	92.9	260.9
16 (f) (g)	1.0268 1.0258	104.8 102.7	6.0 15.3	85.55 81.85	111.6 109.1	6.4 16.2	3.83	1299.6 1365.5	74.5 202.9	11644 12522	65.9	128.4	878	75.1	146.2	296.4
17 (f) (g)	1.0263 1.0261	102.1 100.3	9.8 19.7	84.50 80.00	108.6 106.3	10.4 20.9	4.38	1292.1 1380.2	123.8 271.4	11894 12990	88.1	147.6	1096	80.4	134.7	295.5
18 (f) (g)	1.0258 1.0250	105.4 103.5	10.8 20.0	84.20 79.15	112.2 109.7	11.5 20.2	3.44	1346.5 1444.7	137.6 266.3	12002 13175	98.2	128.7	1173	83.7	109.7	276.8
Normal																
FWS (f) (g)	1.0269 1.0268	101.3 101.3	5.7 4.7	89.00 89.15	108.3 108.3	6.1 5.0	$\infty$	1200.2 1194.9	67.1 52.2	11084 11032	5.3	12.0	52	102.1	231.5	435.1
FSW (f) (g)	1.0270 1.0271	102.2 102.9	5.1 2.9	85.05 85.40	108.8 109.5	5.5 3.1	3.43	1270.4 1267.6	64.0 35.9	11680 11572	2.8	28.1	109	25.8	259.4	311.0

ments were made by means of the Stadie-Sunderman apparatus (10).

# CALCULATION OF RESULTS

The concentrations of glucose and chloride in serum before and after the glucose administration have been expressed as moles per liter of serum; as moles per kilogram of serum water; and as moles per kilogram of dry residue exclusive of the glucose and chloride. It is the second of these, the molality of a solute in serum which is most simply related to changes in the osmotic equilibrium, thermodynamic potential, or activity of the solute. If it be assumed as a plausible approximation for these experiments, that, excluding the glucose and the chlorides, the remaining solids of the serum remain constant during the ninety minute period of the experiment, then from the ratios of glucose, chloride, and water to solids the percentile change in the total quantities of these three components may be calculated.

The following equations were employed in our calculations (those for glucose being analogous to those for chloride).

## SYMBOLS

- Sp = specific gravity at 20° C.  
 H<sub>2</sub>O = water in grams  
 S = solids in grams  
 Gl = glucose in millimoles  
 Cl = chloride in millimoles  
 L = liters of serum  
 K = kilograms of serum  
 [ ] = concentration per kilogram of water  
 R = residual solids in kilograms. (Solids exclusive of glucose and chloride as NaCl)  
 $\pi_{ex}$  = osmolal concentration of glucose and chloride in the fluid calculated as exchanged  
 subscript f = fasting  
 subscript g = after glucose

$$\frac{Cl/L}{Sp} = Cl/K; \quad \frac{H_2O/L}{Sp} = H_2O/K = 1000 - S/K, \quad (1)$$

$$\frac{1000 Cl/K}{1000 - S/K} = [Cl], \quad (2)$$

$$Cl/R = \frac{1000 Cl/L}{Sp (S/K) - 180 Gl/L - 58.45 Cl/L}, \quad (3a)$$

$$H_2O/R = \frac{Sp \times 10^6 - Sp (S/K) \times 10^3}{Sp (S/K) - 180 Gl/L - 58.45 Cl/L}. \quad (3b)$$

Transference of H<sub>2</sub>O, Cl, and Gl to the serum after glucose administration.

$$\Delta Cl/R = (Cl/R)_g - (Cl/R)_f, \quad (4a)$$

$$\Delta H_2O/R = (H_2O/R)_g - (H_2O/R)_f. \quad (4b)$$

Concentrations of Cl and Gl (expressed as mM. per kilogram of water added) in the solution entering serum after glucose administration are as follows:

$$\Delta Cl/(\Delta H_2O \times 10^{-3}) = \frac{1000 \Delta Cl/R}{\Delta H_2O/R}. \quad (5)$$

Estimated osmolal concentration of chloride and glucose in the fluid calculated as exchanged ( $\pi_{ex}$ ) assuming 1.9 as the osmolal equivalence of a mole of electrolyte.

$$\pi_{ex} = 1.9 \Delta Cl/(\Delta H_2O \times 10^{-3}) + \Delta Gl/(\Delta H_2O \times 10^{-3}). \quad (6)$$

## RESULTS

The data pertinent to this paper have been recorded in Table I.

### *Relationship between the concentrations of glucose and chloride in serum on a water basis*

From calculations made from the data in this paper and in a previous paper (11) there was no significant correlation between the concentration of glucose and chloride in either the serum or corpuscles in the blood of different patients. However, following the ingestion of glucose in diabetic patients there was a striking correlation between the *change* in the concentrations of glucose and chloride in the serum when these were expressed on a water basis.

In the serum of each of the diabetic patients following the administration of glucose there was a decreased concentration of chloride as well as the anticipated increased concentration of glucose. The increase in the glucose concentration in moles per kilogram of water was accompanied by a decrease in the chloride concentration in moles per kilogram of water in the mean ratio of 3.77  $\pm$  0.45 to 1.

In Figure 1 the increase in the milliosmolal concentration of glucose in the serum is plotted against the decrease in the milliosmolal concentration of chloride. The statistically calculated regression line of chloride on glucose is:

$$-\Delta \pi_{Cl} = 0.369 \Delta \pi_{glucose} + 1.50.$$

If the osmolal decrease in concentration of chloride were equal to the osmolal increase in glucose the curve would follow the dotted line. The increase in the concentration of glucose when greater than about 3 mM. per kilogram of water is thus not entirely compensated for osmotically by the decrease in the concentration of chloride.

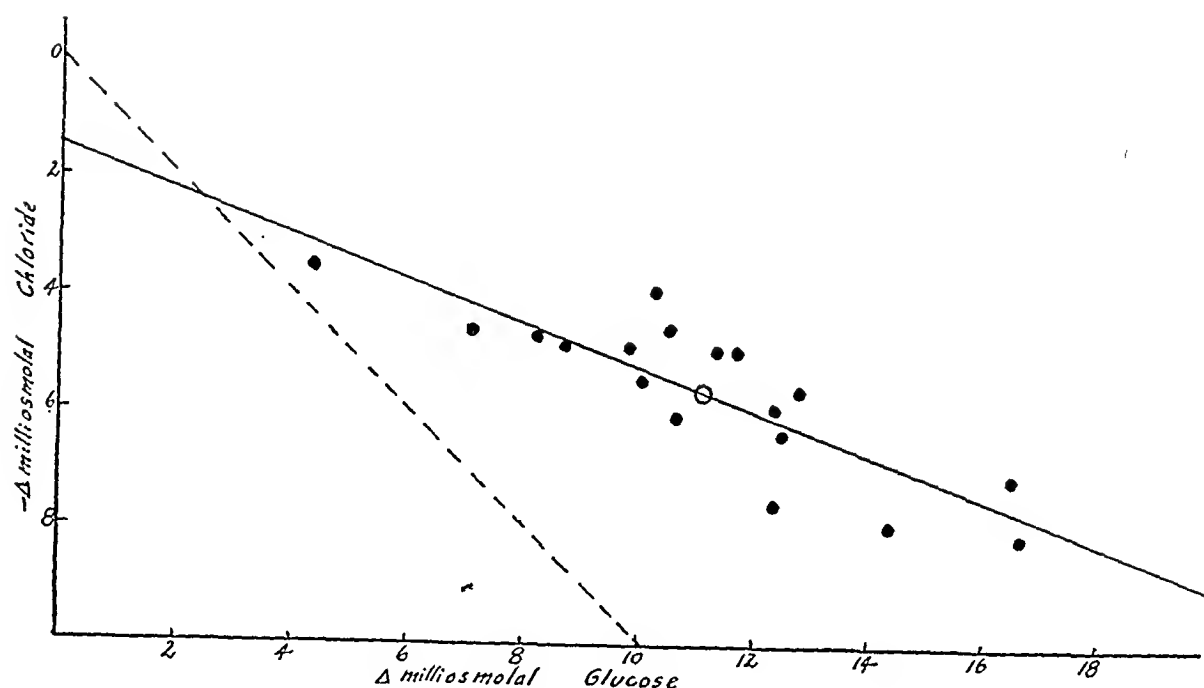


FIG. 1. INCREASE IN OSMOLAL CONCENTRATION OF GLUCOSE PLOTTED AGAINST THE DECREASE IN OSMOLAL CONCENTRATION OF CHLORIDE.

TABLE II  
Effect of ingestion of glucose by diabetic individuals on the total base and freezing point of serum

Case	Freezing point	Total base	Glucose	H <sub>2</sub> O
	° C.	m. Eq./kgm. H <sub>2</sub> O	mM./kgm. H <sub>2</sub> O	grams/kgm. serum
19 (f) . .	- 0.544	158.8	7.73	901.45
(g) . .	- 0.552	157.5	12.66	904.65
20 (f) . .	- 0.527	156.1	7.66	909.40
(g) . .	- 0.533	151.0	17.19	913.65

In Table II are given concentrations of total base and glucose in relation to the water in the serum of two diabetic patients. With increase of serum glucose the total base was diminished in the serum of both of these patients. It would appear thus that the decrease in the concentration of chloride was probably accompanied by a decrease in the concentration of total base.

#### *The composition of the serum after ingestion of glucose*

The concentrations of glucose and chloride in each sample of serum obtained before and after the administration of glucose are given in Table I and are expressed per unit of volume, per unit of water, and per unit of residual solids. Although the concentration of chloride per liter and

per kilogram of serum water was decreased in all of the cases following glucose, the amount of chloride per kilogram of residual solids in the serum, with the exception of Case 5, was increased. In Case 5, as in the others, there was a greater amount of glucose and water present in the serum after the administration of glucose than before.

In the upper part of Figure 2 is given the percentile increase in the water of the serum per kilogram of solids. In the lower part of the graph the open columns represent the osmolal concentration of the increment of glucose and the hatched columns the osmolal concentration of the increment of chloride calculated as if they were in the increment of water. The analyses indicate that in 17 of the 18 cases, the increment in chloride was from 5.4 to 103 m. Eq. per kilogram of increment of water. In Case 5 the calculation indicates a slight loss of chloride from the serum. The estimated osmolal concentration of the fluid increment with both chloride and glucose included was on the average higher than that of the fasting serum. This is consistent with the observed increase in the freezing point depression in the serum after glucose ingestion (Cases 19 and 20).

In Table I and Figure 2 are given the measurements obtained from similar analyses in two normal individuals following the ingestion of glu-

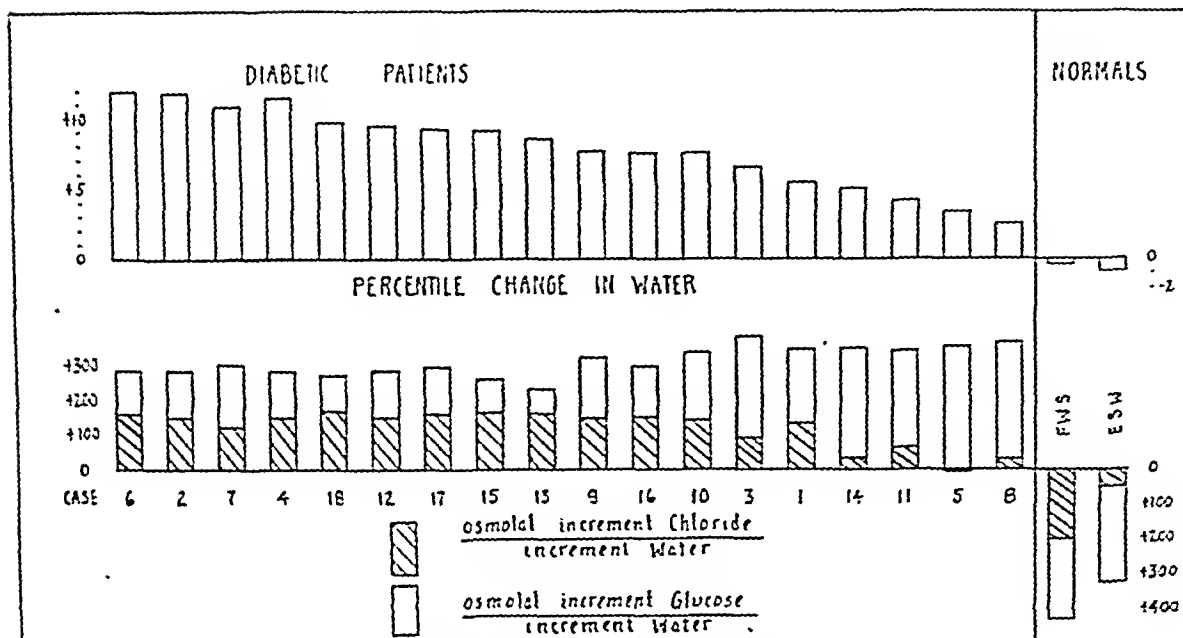


FIG. 2. CHANGES IN SERUM AFTER GLUCOSE INGESTION.

cose. If the same assumption be made that there is no change in the total solids (excepting glucose and chloride) in the serum of the normal individuals after the ingestion of glucose then it will be observed that there was an outflow of water as well as of glucose and of chloride. The changes of volume and composition of the serum in normal individuals were just the reverse of those observed in the diabetic patients.

While there are many studies in the literature giving changes in concentration of solutes per liter of serum following hemorrhage, plasmapheresis, intravenous injection of hypotonic or hypertonic solutions, etc., few studies contain in addition, measurements of specific gravity and dry weight. It is, therefore, impossible to calculate from them the changes of solute per kilogram of water or to estimate the sign or the approximate ratio,  $\Delta \text{solute} / \Delta \text{H}_2\text{O}$ .

#### SUMMARY

Blood was removed from fasting diabetic patients before and  $1\frac{1}{2}$  hours after the ingestion of glucose. The increase in the concentration of glucose in the serum as moles per kilogram of water was accompanied by a decrease in the concentration of chloride in the same units in approximately the ratio of 3.8 to 1.

The assumption was made that the amount of solids in the serum, excepting glucose and chloride, remained constant during the brief period of an experiment and the amounts of glucose, chloride and water present in the serum before and after the administration of glucose were calculated in relation to these residual solids. The results of these calculations indicated that after the ingestion of glucose there was not only an increase in the total quantity of glucose in the serum but also an increase in the total quantities of chloride and water present. The increase in the total quantity of chloride was from 0 to 103 m. Eq. per kilogram of added water. The increment in glucose plus chloride calculated in relation to the increment of water gave, on the average, a value representing a higher osmole concentration than that of the fasting serum. The final results obtained after the ingestion of glucose by diabetic individuals, therefore, were an increase in glucose concentration, a decrease in chloride concentration, an increase in the osmotic pressure, and an increase in the total quantities of glucose, chloride, and water of the serum.

In the serum of the diabetic patient the disturbance resulting from the uptake of glucose is distributed among at least three other variables: serum volume, osmotic pressure, and chloride con-

centration. The experiments illustrate the fact that a change in concentration induced with respect to a single component, such as glucose, tends to disturb the concentration of other components of the serum and to induce transfers of some of them to or from the serum.

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# THE WATER AND BASE BALANCE OF THE BODY<sup>1</sup>

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## INTRODUCTION

A satisfactory method for the quantitative estimation of changes of hydration of the body is not available. Measurement of obvious fluids of the intake and output is of limited value. The method of determination of total water exchange proposed by Newburgh (1) has proved unsatisfactory in our experience (2). An approach to the problem through study of the electrolyte metabolism has the advantage that it gives information not only concerning the exchange of fluids between body and environment but also concerning the distribution of these fluids within the body.

That base (excluding calcium) and water are lost by the body in approximately the proportions in which they appear in the plasma was demonstrated by Gamble, Ross and Tisdall (3) from studies of fasting epileptic children. They suggested the use of this relationship for the estimation of water exchange from determined balances of cations. This procedure presupposes that total base is distributed evenly throughout the entire volume of body fluids and that the concentration of total base in these fluids remains constant while the volume of fluids changes. This need not be true, of course, for individual cations. Gamble's concept has gained wide acceptance and has served to add much to our knowledge of the interrelationship of the metabolism of base and of water.

The application of improved analytical methods to the determination of serum base has demonstrated that variations of one or two per cent are common, and that much wider variations occur under unusual circumstances. Comparisons by direct analyses of tissues (4), transudates (5, 6, 7, 8), and red blood corpuscles (9, 10, 11) with serum have shown, as might be inferred from the results of Gamble's experiments, that concentrations of total base per unit of water are approximately alike in all these media. The concentration

of base in the water of serum differs, however, slightly, but definitely, from that in the water of transudates or of red blood cells. Its relation to the concentration in other divisions of the body water has not been determined with sufficient accuracy to permit discussion. Whatever this may be, there is considerable evidence that a change of the concentration of base in any portion of the body water elicits changes of like direction and degree in all other portions. Klinghoffer (12) has shown that when water is added to human blood *in vitro*, the added water is redistributed almost immediately in such a manner that cell and serum water are increased nearly proportionally. Since it has been shown previously that under these conditions no base traverses the cell membrane (13, 14), the inference that the base of cellular water falls nearly in direct proportion to that of the serum seems warranted. In similar experiments in which salts of sodium and potassium were added to blood, sufficient water was transferred from the cells to the serum to keep the concentration of total base per unit of water in cells and serum approximately equal (14). When urea or glucose solutions were added to blood, the water of cells and of serum increased nearly proportionally, the added solutes distributing themselves evenly throughout all the water present, without any exchange of base between cells and serum (12). When hypertonic solutions of sucrose, to which the cell membranes are impermeable, were added, on the contrary, water was drawn from the cells, and the concentration of base in the water of the cells rose above that in the water of the serum (12). These findings are in accord with the theory of osmotic pressure which demands that only those solutes to which the cell membrane is impermeable exert an osmotic influence upon the distribution of water between cells and serum. In the body the total electrolyte concentrations can be measured by the concentrations of the inorganic bases which are apparently unable to traverse cellular membranes

<sup>1</sup> Part of the expense of this investigation was defrayed by a grant from the Ella Sachs Plotz Fund.

and make up the major portion of the osmotically effective substance of the fluid media. Changes in the concentration of base in any portion of the body water should, then, be compensated by like changes in the other portions, if osmotic equilibrium is to be maintained. In several experiments after large changes in the concentration of base in the serum of man *in vivo*, the volume of water in the cells has changed proportionally in the opposite direction (15), conforming to the results of the *in vitro* experiments cited above. There is no direct evidence that the concentration of base in the water of tissues other than the red blood cells varies directly with that of serum, but from theoretical considerations one would expect this to be true if the isotonicity of the body fluids is to be maintained, unless non-electrolytes to which the membranes are not permeable are quantitatively significant or unless there is change of pH and hence of base bound by protein. Schechter (16) and Schechter, Cary, Carpentieri and Darrow (17) have found that, following the intraperitoneal injection of various fluids in dogs, there is a fairly rapid redistribution of water and electrolytes so that the peritoneal fluid approaches the relationship to serum which is ordinarily found to exist between transudates and serum. In similar experiments, Yannet and Darrow (11) found that measures which changed the concentration of base in the serum caused inverse variations in the size of the red blood corpuscles. Direct analyses of whole blood and serum in these experiments showed that, with few exceptions, the actual amount of base in the cells remained unchanged, but that the changes of volume were sufficient to equalize the concentrations of base in the water of cells and serum.

If it is true that changes of the concentration of base are distributed over a large volume of fluid, even slight changes in concentration may be quite significant in attempts to relate balances of water and base. For example, in a subject weighing 50 kgm., of which about 35 kgm. is water, a change of two milliequivalents in the concentration of base would allow the gain or loss of  $35 \times 2 = 70$  m. Eq. of base without change in the volume of fluid in the body. Conversely, a change of 2 m. Eq. in the concentration of base, if this were originally 150 m. Eq. per liter, would be produced by a change in the volume of body fluids

of  $35 \times \frac{2}{150} = 0.467$  liters without either gain or loss of base by the body. Such phenomena may explain the apparent lack of correlation between balances of water and base which have been observed occasionally in this laboratory and elsewhere. Even small changes of concentration cannot be dismissed from consideration in such studies, as has frequently been done, merely because methods adequate to measure such variations have not been available.

#### Total water exchange

If base does tend to distribute itself throughout the fluid media of the body in definite and relatively constant proportion to water, it should be possible to estimate body water exchange from the balances and changes of concentration of base in serum by the following formula:

$$W_1 B_1 + b = W_2 B_2. \quad (1)$$

in which  $W_1$  and  $W_2$  represent the volume of water in the body at the start and at the conclusion of the period of study, respectively;  $B_1$  and  $B_2$ , the corresponding concentrations of Na + K in the water of serum; and  $b$ , the net gain or loss of Na + K by the body. This equation may be written

$$W_1 B_1 + b = B_2 (W_1 + \Delta W),$$

in which  $\Delta W = W_2 - W_1$ , or water exchange. Solved for  $\Delta W$ , this becomes

$$\Delta W = \frac{b + W_1 \Delta B}{B_2}, \quad (2)$$

in which  $\Delta B = B_1 - B_2$ , the change of the concentration of base in the water of the serum. If the concentration of base does not change, the equation is simplified to:

$$\Delta W = \frac{b}{B}, \quad (3)$$

which represents essentially Gamble's method of calculation. Na + K has been used rather than total base because the other bases, calcium and magnesium, are found in the water of the body in relatively small amounts and exert an osmotic effect that is small as compared with their acid-combining power because they are bivalent and partly in undissociated combinations with protein.

This is certainly true of serum, transudates and red blood corpuscles. Tissue cells, however, contain a considerable amount of magnesium, neglect of which may conceivably introduce error into calculations. In prolonged fasting (3, 18) the ratio

$\frac{\text{Mg}}{\text{K} + \text{Mg}}$  in the excreta, expressed in millimols, is almost identical with that in muscle, 0.097 (4).

It is, therefore, possible that ten per cent of the osmotic effect of the base of cells is contributed by magnesium. In the one experiment here reported in which magnesium balances were determined the largest change noted was a retention of 12 millimols in a four-day period.

Equation 2 can be solved for total water exchange providing a value for the initial volume of fluid,  $W_1$ , can be found. It has been customary in metabolic work to assume that approximately 70 per cent of the weight of the body is water. The water content of normal rats, rabbits and dogs has been found by direct analysis to approximate this value (19, 20). Measurements of osmotic changes in the serum following the injection of hypertonic salt solutions by Hetherington (21) indicated that on the average 59 per cent of the body weight of cats was composed of water which was available as solvent. It should be possible, in short studies on subjects in whom large changes of body water and base are induced by various measures, to determine the initial volume of fluids from Equation 2 by assuming that observed changes of body weight represent water exchange. This unfortunately has not proved of value because the changes in concentration must be extremely large to overbalance analytical errors. In a single experiment with forced urea feeding, the urea was found to distribute itself through approximately 70 per cent of the body weight of a normal subject (22). In the calculation of water exchange by Equation 2, the initial water content of the body will be taken to be 70 per cent of the initial body weight, with the understanding that this is merely an approximation, serving to correct only roughly for changes in the concentration of base in the water of the body.

#### *Changes in the volume of extracellular fluids*

There is much evidence to support the view that almost all of the sodium of the body is confined

to the extracellular fluids, and that the same is true of chloride, if the red blood corpuscles are excluded from consideration. This evidence has been summarized briefly by Peters (23). If this be true, extracellular water exchange ( $\Delta E$ ) may be calculated from sodium metabolism as follows:

$$\text{Na}_1 E_1 + b_{\text{Na}} = \text{Na}_2 E_2,$$

where  $\text{Na}_1$  and  $\text{Na}_2$  represent the average concentrations of Na in extracellular water at the beginning and at the end of the period of study;  $E_1$  and  $E_2$  represent the volume of extracellular fluids at corresponding times; and  $b_{\text{Na}}$  represents the Na balance. By substituting  $E_1 + \Delta E$  for  $E_2$  in the above equation, it may be solved for  $\Delta E$ .

$$\Delta E = \frac{b_{\text{Na}} + E_1(\Delta \text{Na})}{\text{Na}_2}. \quad (4)$$

Likewise if the Cl of the red blood corpuscles is neglected,<sup>2</sup>

$$\Delta E = \frac{b_{\text{Cl}} + E_1(\Delta \text{Cl})}{\text{Cl}_2}. \quad (5)$$

While it is known that the concentration of Na in the water of transudates is slightly lower than that of serum water and that of Cl slightly higher (5, 6, 7, 8), changes in the concentration of either Na or Cl of serum are reflected in similar changes in their concentrations in transudates. Little error will therefore be introduced into the calculations if, for the average concentrations of Na and Cl in extracellular water, their respective concentrations in serum water are used. A single unknown,  $E_1$ , remains.

Theoretically if Equations 4 and 5 are both accurate expressions of extracellular water exchange, it should be possible, by equating the two, to solve for the initial volume of these fluids.

<sup>2</sup> If 7 per cent of the body weight is assumed to be blood, with a cell volume of 40 per cent and cell water of 70 per cent, the water of the red blood corpuscles becomes  $7 \times 0.4 \times 0.7 = 2$  per cent of the body weight. The concentration of chloride in this water is considerably lower than that in the serum water (approximately 75/110), and if other factors remain constant, changes of concentration occur in the same ratio. On the basis of the above values, the red blood cells of a 50 kgm. man contain only about 75 m. Eq. of Cl, an insignificant amount in comparison with the Cl of the interstitial fluids.



Practically this procedure has been impossible because errors well within the limits of analytical accuracy may prove of overwhelming significance. It is possible that in experiments specially planned to produce large discordant changes in the concentrations of Na and Cl this method may yet prove feasible. From the values for base in muscle, obtained by Katz (4), by assuming that all the Na is contributed by interstitial fluid, it has been calculated that extracellular water made up 20 per cent of the muscle weight (23). These figures are corroborated by recent analyses of dog muscle made by Hastings (24). Eggleton (25, 26) has shown that certain solutes diffuse through all the water of the frog muscle, while other substances diffuse through only 20 to 30 per cent of the water of vital frog muscle, presumably the extracellular portion. After rigor mortis has set in this selective permeability disappears. Crandall and Anderson (27) have found that after the intravenous injection of NaCNS, the CNS radical distributes itself through approximately 24 per cent of the body weight of normal man. These authors suggest this procedure as a measure of the "state of hydration" of the body. In tissues, analyses revealed little enough CNS to be attributed solely to the interstitial fluid included with the cellular substance. It seems probable, then, that this procedure measures roughly the volume of the interstitial fluids plus the water of the red blood corpuscles. If this is true, the extracellular fluid volume in their series would be about 22 per cent of the body weight. The salt was found to enter the gastro-intestinal secretions so that if any appreciable amount of these secretions were formed during the experiments, the true value for the volume of extracellular fluid would be even lower than this. Preliminary studies of the distribution of intravenously injected sucrose in man conducted in this Department indicate that the volume of the interstitial fluids normally approximates 20 per cent of the body weight. It seems justifiable, on the basis of the foregoing evidence, to use as a rough approximation of extracellular fluid volume in Equations 4 and 5, 20 per cent of the body weight. The results of the calculations are but little altered if 15 or 25 per cent is used instead.

The data from 10 experiments conducted in this Department are available for evaluation of

the formulae described for the study of water exchanges. These data are suitable for this purpose because they satisfy the following criteria (except in the last two experiments which will be discussed separately):

1. The body tissues presumably changed little because the food taken approximated the energy requirements.
2. Water exchanges were sufficiently large to permit the assumption that, within the requisite limits of error, water exchange was equal to the change of body weight.
3. Digestive disturbances were absent. Diets were completely taken except in Experiments 7 and 8 in which a little food was refused. No food or fluids had been taken for at least 8 hours before the start or end of an experiment.
4. There was no evidence of sensible perspiration.

#### EXPERIMENTAL PROCEDURE

No food, drink or medication was given for at least 8 hours, and usually 12 hours, before a period of study was started or concluded, except in the subjects with uncontrolled diabetes insipidus in whom water was withheld for a shorter period. The subject was weighed at the beginning and at the end of each period of study. A silk balance capable of weighing  $\pm 5$  grams was used. Blood for analysis was drawn before the first and after the second weighing in experiments consisting of only one period. When an experiment comprised more than one period, blood drawn was included in the output for the period in which it was drawn in the calculation of balances and insensible perspiration. Urine voided just before the first weighing was discarded; all the subsequent voidings, including one just prior to the second weighing, were combined for analysis. Carmine capsules were used to separate the stools. Salt-poor mixed diets were prepared in the diet-kitchen except in Experiment 5, in which the diet consisted only of weighed portions of milk, cream, shredded wheat, sugar and salt. In Experiments 6 and 8, the diets used in each period were identical in all respects, thus decreasing the possibility of error through variability of diets.

In experiments on diseased subjects, detailed supervision was required to insure the collection of all excreta and the accurate measurement of all ingesta. No pains were spared to gain the cooperation of patients, nurses and orderlies. Water was supplied from the laboratory in weighed thermos bottles. The subjects' tastes were consulted before preparing the diet in order to lessen the probability of refusals. Corrections for the small amounts of food refused by the subject of Experiment 8 were made by the use of the tables of Sherman (28). In Experiment 7, food equal to that refused was removed

from the duplicate diet before it was prepared for analysis. The vomitus present in Experiment 10 was analyzed for electrolytes. Parenteral fluids, used only in Experiments 9 and 10, were weighed, and portions saved for analysis. Urine was collected at the bedside in a three liter bottle containing a crystal of thymol and about one gram of solid benzoic acid. Stools were immediately sent in the bed pans to the laboratory refrigerator. All stools were weighed, but those preceding the appearance of the carmine were not saved for analysis. About 100 grams of water and about 2 grams of benzoic acid were added as preservatives to the stools after they had been weighed. The mixture was kept in the refrigerator.

Anaerobic precautions were observed in collecting blood for analysis. Serum was obtained from blood clotted under oil. Protein was determined by the macro-Kjeldahl method with the aid of superoxol in the digestion; chloride by the method of Van Slyke as modified by Eisenman (29); sodium and potassium by the methods of Barber and Kolthoff (30) and Shohl and Bennett (31) respectively, with the modifications described by Hald (32); total base by a modification of the method of Stadie and Ross (33), with titration of the precipitate, except in Experiment 8 when the gravimetric procedure of Hald (32) was employed. In the shorter experiments, the sera were analyzed together at the end of the experiment, thus eliminating the effect of variable blanks and reagents on the comparability of the analytical values.

Urine N was determined by the macro-Kjeldahl method; urine Cl by the Harvey modification (34) of the Volhard method; Na and K by methods similar to those used for serum (to be published). A generous portion of the urine, including some of the solid benzoic acid contained in it, was saved in a glass-stoppered bottle in the refrigerator to provide for repetition of analyses if these proved necessary. Urine so preserved was found to have an acid reaction and an unchanged chloride concentration after one month.

The stools from each period were transferred with the aid of water to a weighed wide-mouthed three liter bottle, mixed for an hour with a mechanical stirrer, weighed, and a portion saved for analysis. Weighed aliquots were analyzed for Cl by the method used for serum; Na and K by the methods described for urine; and N by the Kjeldahl method with the aid of superoxol in the digestion. In the later experiments wherever K was present in excess of Na, as was the case in all of the diets, most of the stools and some of the urine specimens, Na was determined after K had been removed with chloroplatinate as described by Hald (32).

Duplicate diets prepared in the diet kitchen were weighed. The fluid portions were transferred to a wide-mouthed 3 liter bottle. The remainder, after it had been passed through a meat-grinder, was added to the fluids. The whole was mixed with a mechanical stirrer for an hour, and a portion, to which about 1 gram of benzoic acid was added, was saved for analysis. Weighed aliquots were analyzed by the methods described for stools, it being assumed that the aliquots so obtained were repre-

sentative of the entire original weight of the diet, this weight being therefore used in the calculations. On one occasion, when the diet was reweighed in the mixing bottle at the end of the procedure, it was found to have lost exactly one per cent of its original weight. The effect of this loss is insignificant, particularly since it probably has approximately the same composition as the whole. An alternative method of preparation, in which the containers and grinder were washed quantitatively with water and the entire mixture weighed before aliquots were taken, gave almost identical results in the one instance in which the comparison was made. The uniformity of the food mixture has been repeatedly ascertained by analyses of aliquots taken from the top and bottom of the mixture. Nitrogen analyses on diets so prepared usually give results 5 to 10 per cent higher than the values predicted from the diet tables. This may be due to the fact that nitrogen may be lost by volatilization if materials are dried preparatory to analysis, the procedure employed for most of the analyses from which the tables were compiled. The essential correctness of our analytical values for N of food is confirmed by the exact balances which have been obtained in prolonged studies of normal individuals with constant adequate intakes. The diets usually used in these experiments contained of the order of 60 m. Eq. of Cl, 50 m. Eq. of Na and 100 m. Eq. of K daily. The constantly noted excess of Cl over Na gave rise to some misgivings. The results of Cl analyses by the method of Sunderman and Williams (35), however, agreed with those obtained by our method, and the values for sodium plus potassium determined separately checked satisfactorily with those secured by their determination as combined sulfates. It was found that analyses for Na and K in excreta and ingesta yielded more consistent and generally higher results when small aliquots (about 5 grams) were asked than when larger aliquots were used. Because of the difficulty of completely ashing the larger aliquots employed earlier (20 to 40 grams), these ions were probably lost by volatilization or incompletely recovered. By using small aliquots and observing all the precautions described by Hald, it is felt that accuracy within  $\pm 3$  m. Eq. daily may be attained in the determination of either Na or K of the urine or food, although a greater error undoubtedly was present in the earlier experiments when the methods were as yet poorly developed. It has been possible to reproduce diets which vary only slightly in the amounts of Na and K which they contain.

#### EXPERIMENTAL SUBJECTS AND TREATMENT OF EXPERIMENTAL RESULTS

The subject of Experiment 1 was a male with hypertension who, during the experimental period of 8 days, gained 490 grams in weight spontaneously. The subjects of Experiments 2 and 3 presented the nephrotic syndrome. The former had a spontaneous diuresis during the experimental

period of 4 days. In the latter water storage was induced by the administration of sodium chloride. The subject of Experiments 4 and 5 was a normal male. In the first experiment a slight water loss was induced by the administration of 40 grams of urea daily. In the second, marked dehydration was produced by the administration of 180 grams of urea with limited fluid intake during the first 24-hour period, and rehydration was partially accomplished during the second period by the administration of added water and salt. The subjects of Experiments 6 and 7 were patients with diabetes insipidus who were allowed water *ad libitum*. In Experiment 6,<sup>3</sup> the same low-salt diet was given in each period. During the first period, 0.5 cc. of pituitrin was given three times daily; during the second no pituitrin was given. In Experiment 7 the diet was approximately the same each day except that added salt was given during the last 2 periods. One cc. of pituitrin was given four times daily during Periods 2 and 5. The subject of Experiment 8 had rheumatic heart disease with moderate cardiac decompensation and rheumatic nephritis with marked renal insufficiency. Large amounts of water were administered in both periods. The food was identical during the 2 periods, but 35.5 grams of NaCl were given in addition to the diet during the second period.

Results with the method of Newburgh and Johnston for the determination of water exchange of the body, which, it had been hoped, would serve as a check upon these calculations, proved disappointing (2). Under the conditions of the present experiments, in which diets approximate energy requirements and in most cases were taken for many days preceding the experiments, little error can be introduced by assuming that observed changes of body weight represent exchanges of fluid only. Where the periods are short and the weight changes large this is especially true. In Experiments 9 and 10, a more difficult situation exists. In the former, a girl of 17 years, with vomiting probably of neurotic origin, had retained very little food for a week prior to the experiment. During the 40 hour experiment nothing

was given by mouth and no nausea or vomiting occurred. The food intake was restricted to 90 grams of glucose and 33.3 grams of NaCl given parenterally. The patient was extremely cooperative and quiet. Because of the previous starved condition the metabolism must have been low and the glycogen stores probably suffered little or no further depletion during the experiment. The urinary N indicated a protein metabolism of 60 grams. The energy requirement of this small inactive subject would probably be more than covered by the additional loss of 200 grams of fat. The error incurred by the assumption that water exchange equals the body weight change plus about 260 grams to replace the tissue losses must be very slight. The subject of Experiment 10, a patient with anuria resulting from a suicidal attempt with mercuric chloride, was given only saline and glucose solutions parenterally and water and sodium bicarbonate by mouth. During the second day of the experiment he felt somewhat nauseated and vomited once following the ingestion of water, but during the remainder of the experiment, suffered no discomfort. During the 3d and 4th days urine secretion was reestablished. The insensible perspiration, which varied widely from day to day, averaged 1504 grams per day. If the normal relationship between insensible loss and metabolism is assumed to exist, this would indicate a metabolism of over 3300 calories per day. A considerable depletion of glycogen must have occurred during this period of virtual starvation. At any rate it is reasonable to assume that a loss of over one kilogram, and not more than two kilograms of body tissue must have occurred during this 4 day experiment so that water gained must have exceeded the weight gained by that amount.

#### PRESENTATION OF RESULTS

The essential data are presented in Table I. The concentrations of Na, Cl and protein in the serum are given in all cases; those of K and total base whenever they are available. The reliability of the base values in the first 4 experiments must be questioned on the basis of internal evidence and errors of chemical analysis which were discovered later. The differences between Na and total base in the first and fourth experiments and in the final examinations in Experiments 2 and 3 are too

<sup>3</sup> For the opportunity to study this patient the authors are indebted to Dr. Daniel C. Darrow, Assistant Professor of Pediatrics.

TABLE I  
Essential data

Experiment number	Subject	Date	Duration	Body weight	Concentrations in serum						Intake				Output			
					Na	K	Total base	Cl	Protein	Water*	K	Na	Cl	N	K	Na	Cl	N
					m. Eq.	m. Eq.	m. Eq.	m. Eq.	per cent	per cent	m. Eq.	m. Eq.	m. Eq.	grams	m. Eq.	m. Eq.	m. Eq.	grams
1...	J. McC.	1930	8	67.55	139.6	6.2	117.7	101.3	7.35	93.4	614	675	719	92.0	545	653	709	89.5
2...	P. F.	1930	4	68.01	140.0	3.5	147.0	102.7	6.85	93.7								
				61.31	125.5	6.5	145.5	105.5	4.05	95.7	484	191	273	85.2	334	343	492	74.0
3...	D. C.	1931	10	59.51	136.0	5.5	145.1	105.0	4.15	95.6								
				63.42	138.0	5.5	152.2	106.1	6.11	91.0	638	1292	1374	92.2	623	1109	1099	63.9
				65.23	135.5	5.4	144.8	103.5	5.83	91.4								
4...	P. L.	1932	6	74.92	143.2	4.3	101.6	7.22	93.4	411	466	523	203.5	410	478	565	197.3	
				74.43	145.7		102.0	7.25	93.4									
5...	P. L.	1933	1	78.90	137.5	4.6	101.0	6.53	93.9	79	42	53		85	253	256		
				77.03	137.2	4.6	101.6	7.19	93.5	70	379	353		93	183	216		
				77.92	132.5	4.8	103.5	7.03	93.6									
6...	J. V.	1933	2	26.55	129.9		96.0	6.50	92.5	150	69	104	19.2	114	67	69	16.2	
			2	27.28	129.9		97.2	7.50	93.3	150	69	104	19.2	154	35	94	17.9	
				26.17	136.1		99.6	8.14	92.8									
7...	M. C.	1933	2	73.46	147.2		117.0	7.24	93.4	150	51	87	16.0	87	95	102	20.7	
				74.54	135.5		111.1	6.82	93.7	165	60	91	20.2	123	81	85	18.0	
				76.30	139.2		103.6	6.27	91.1	109	65	69	20.0	141	123	194	20.1	
			1	74.15	132.5		112.1	6.95	93.6	67	376	319	10.9	97	139	146	10.0	
			1	74.25	142.2		114.0	6.78	93.8	72	331	355	10.4	69	150	127	8.4	
				77.44	133.4		107.4	5.81	91.4									
8...	J. M.	1933	4	49.50	126.4	4.3	137.0	92.1	5.35	91.8	232	205	237	41.0	172	254	231	31.3
			4	51.10	119.4	5.3	132.1	95.4	5.51	94.7	251	513	845	41.3	147	195	219	29.7
				51.41	128.7	5.7	141.3	108.6	5.65	91.6								
9...	P. C.	1933	1.5	46.2	125.0		83.7	8.72	92.1	0	570	570	0	27	25	6	10.1	
				49.7	133.5		98.2	6.09	91.3									
10...	F. T.	1932	4	68.91	121.0	6.5	132.9	91.6	5.32	94.8	0	932	828	0	65	321	335	7.2
				67.29	126.6	6.1	139.3	95.5	5.68	91.6								

\* Serum water was not determined but was calculated from serum protein by the formula  $98.57 - 0.709$  (Protein), derived statistically by Eisenman and Peters (44).

small to allow for the expected amounts of bases other than Na. This is probably due to the fact that in precipitating the sodium salt in the early experiments as little water as possible was used in dissolving the ash instead of the fixed proportion of water to reagent recommended by Kolt-hoff, thus yielding results for Na which were too high. In Experiment 1 there must be an error in the K analyses since the wide variation is at odds with the constancy of Na, total base, and Cl. In Experiment 2, a large rise in Na occurs without any comparable rise in total base. In the third experiment, the observed decreases in Na and total base are disproportionate. In the remaining experiments, errors in the determinations of either Na or Cl greater than 1 m. Eq. per liter are improbable. In all of these the sum of the determined concentrations of Cl, CO<sub>2</sub> and protein in the serum, expressed in terms of base-combining powers, were used to check the observed changes of concentration of base. In Experiments 7, 8 and 9, osmotic pressure, determined by Dr. A. Gilman of the Department of Pharmacology, was found to vary proportionally to base. In Experiment 8, the complete system of base analyses was performed. The results are presented in Table II.

TABLE II  
Data on serum from Experiment 8

Serum	Na	K	Na+K as SO <sub>4</sub>	Ca	Mg	Sum of bases	Total base as SO <sub>4</sub>
	m. Eq.	m. Eq.	m. Eq.	m. Eq.	m. Eq.	m. Eq.	m. Eq.
1	126.4	4.3	130.1	4.4	1.4	136.5	137.0
2	119.4	5.3	123.5	3.7	1.8	130.2	132.0
3	128.7	5.7	134.2	3.4	1.9	140.0	141.3

The recorded outputs of electrolytes and N neglect any loss which may occur through the skin and lungs. Loss of N through these avenues is negligible even when moderate sweating occurs. That appreciable loss of electrolytes may occur through sweating is admitted. Sensible perspiration did not occur in any of these experiments, however. There is much evidence to show that under these conditions electrolyte loss through the skin is negligible. The chloride of urine and stool has been found to approximate very closely that of the intake in a prolonged study on a normal subject who avoided sweating (36), thus making it improbable that the insensible perspiration contained appreciable amounts of Cl. Hancock, Whitehouse and Haldane (37) were able to re-

cover only 0.09 gram Cl and 0.08 gram K from the washings of the skin of a normal subject who had not bathed for one week, but who had not sweated sensibly nor been exposed to warm weather. From this, and from further experiments in which variable amounts of sweating were induced, these authors concluded that, when the loss of water through the skin is small, the water passes through the skin by osmosis without the intermediation of the sweat glands, and the small amount of electrolyte lost is derived from epidermis or from the sebaceous secretions. Viale (38) could recover no appreciable amount of Cl from the skin of the arm at rest until the temperature was raised, thus presumably calling the sweat glands into play. Vasti (39) applied strips of smooth white paper to the washed skin of normal human subjects for a few minutes. He then dipped the paper in 10 per cent silver nitrate and exposed it to the sunlight. By this method the passage of even minute traces of Cl through the skin may be detected. Although loss of water from the skin area could be demonstrated in every case, punctate brown stains, presumably marking active sweat glands, were found in only 5 instances. In these the insensible perspiration was 18 to 72 per cent above the average. It seems reasonable to conclude that where the sweat glands

are inactive, perspiration occurs only through evaporation of water from the superficial layers of the skin which is continuously renewed from below by the resulting osmotic gradient. This entails no loss of salt. Under the conditions of our experiments, the activity of the sweat glands must have been minimal and loss of electrolytes through the skin may be neglected.

In Table III the data are presented in a form which is more convenient for the calculation of water exchanges. The balances of Na + K, Na and Cl are recorded as  $b$ ,  $b_{Na}$  and  $b_{Cl}$  respectively. The concentrations of electrolytes in the water of serum recorded to the nearest milliequivalent, are obtained by dividing the observed concentrations by the water content of the serum. Finally 70 per cent of the initial body weights are recorded as  $W_1$ ; 20 per cent as  $E_1$ . These approximations of the initial volumes of the total and the extracellular water of the body have been used in the calculations for reasons previously discussed.

In Table IV are presented the results of calculation of the water exchanges in these experiments by the formulae proposed above. Total water exchange ( $\Delta W$ ) is calculated from base metabolism with and without correction for changes of concentrations by Equations 2 and 3 respectively. The observed changes of body weight are also

TABLE III  
Data presented for ease in calculation of water exchanges

Experiment	Subject	Electrolyte balances			Concentrations of electrolytes in serum water*										
		$b$	$b_{Na}$	$b_{Cl}$	$B_1$	$B_2$	$\Delta B$	$Na_1$	$Na_2$	$\Delta Na$	$Cl_1$	$Cl_2$	$\Delta Cl$	$W_1$	$E_1$
		m. Eq.	m. Eq.	m. Eq.	m. Eq.	m. Eq.	m. Eq.	m. Eq.	m. Eq.	m. Eq.	m. Eq.	m. Eq.	m. Eq.	m. Eq.	m. Eq.
1...	J. McC.	81	12	19	156	156	0	150	150	0	112	110	2	47	14
2...	P. F.	-49	-149	-129	141	148	-7	134	142	-8	113	110	3	43	12
3...	D. C.	218	183	275	153	151	2	147	145	2	113	112	1	44	13
4...	P. L.	19	-12	-40	158	161	-3	153	156	-3	109	109	0	52	15
5...	P. L.	-217	-211	-203	151	152	-1	146	147	-1	111	109	2	55	16
		168	196	172	152	154	-2	147	149	-2	109	111	-2	54	15
6...	J. V.	38	2	35	145	144	1	140	139	1	104	104	0	19	5
		30	34	10	144	152	-8	139	147	-8	104	107	-3	19	5
7...	M. C.	22	-41	-15	163	153	10	158	148	10	125	119	6	51	15
		8	-24	6	153	143	10	148	138	10	119	110	9	52	15
		-42	-58	-95	143	154	-11	138	149	-11	110	120	-10	54	16
		146	176	173	154	157	-3	149	152	-3	120	122	-2	52	15
		184	181	229	157	146	11	152	141	11	122	114	8	52	15
8...	J. M.	31	-49	6	138	132	6	133	126	7	105	101	4	35	10
		729	625	626	132	142	-10	126	136	-10	101	115	-14	36	10
9...	P. C.	518	545	564	139	142	-3	139	142	-3	91	104	-13	32	9
10...	F. T.	546	611	492	135	140	-5	128	134	-6	97	101	-4	47	13

\* The electrolyte concentrations in serum water are calculated by dividing determined serum electrolyte concentration by serum water content, and recording the results to the nearest unit. A constant value has been assumed for serum potassium when it was not determined by analysis.

TABLE IV  
Water exchanges calculated by various methods

Experiment number	Subject	Total water exchange ( $\Delta W$ ) from		Body weight change*	Extracellular water exchange ( $\Delta E$ ) from		Diagnosis	Intake		Medication
		$b + \Delta B$ (Eq. 2)	$b$ (Eq. 3)		Na (Eq. 4)	Cl (Eq. 5)		H <sub>2</sub> O	Salt	
1...	J. McC.	0.52	0.52	0.49	0.03	0.42	Hypertension	Not restricted	+	0
2...	P. F.	-2.36	-0.33	-1.67	-1.72	-0.85	Nephrosis	"	-	0
3...	D. C.	2.03	1.44	1.81	1.44	2.57	Nephrosis	"	+	0
4...	P. L.	-0.85	0.12	-0.49	-0.37	-0.37	Normal subject	"	-	Urea 40 grams daily
5...	P. L.	-1.79	-1.43	-1.87	-1.54	-1.57	"	Restricted	-	Urea 180 grams
		0.39	1.09	0.89	1.12	1.28	"	Forced	+	0
6...	J. V.	0.40	0.26	0.40	0.05	0.34	Diabetes insipidus	Not restricted	-	Pituitrin
		-0.80	0.20	-1.11	-0.01	-0.05	"	"	-	0
7...	M. C.	3.48	0.14	1.03	0.74	0.63	"	"	-	0
		3.69	0.07	1.76	0.91	1.28	"	"	-	Pituitrin
		-4.13	-0.27	-2.15	-1.57	-2.12	"	"	-	0
		-0.06	0.93	0.10	0.86	1.17	"	"	+	0
		5.18	1.26	3.19	2.45	3.06	"	"	+	Pituitrin
8...	J. M.	1.83	0.24	1.60	0.17	0.45	Nephritis with hyper-tension	Forced	-	0
		2.60	5.13	3.31	3.86	4.22	"	"	+	0
9...	P. C.	2.97	3.65	3.50	3.65	4.30	Psychoneurosis	"	-	Parenteral fluids only
10...	F. T.	2.22	3.90	0.35	3.98	4.35	Poisoning (HgCl <sub>2</sub> )	"	-	Only water and NaHCO <sub>3</sub> per os

\* Except in the last 2 experiments, change of body weight is taken to represent fluid exchange only. For reasons set forth in the text, it has been assumed that the water gain exceeds the weight gain by 0.26 kgm. in Experiment 9 and by 1 to 2 kgm. in Experiment 10.

recorded. Total water exchange calculated from the metabolism of base by Equation 2 is in satisfactory agreement in most cases with the observed weight changes. The most striking exceptions, noted in Experiment 7, will be discussed later. In the first period of Experiment 5,  $\Delta W$  calculated by Equation 2 agrees almost exactly with the loss of body weight; in the second period, however, the calculated exchange is only 0.39 kgm. while the weight increases 0.89 kgm. If the change of concentration of base in the water of serum is assumed to be 1 m. Eq., instead of the observed 2 m. Eq., the calculated  $\Delta W$  becomes 0.74 kgm., which agrees closely with the gain of weight. Since analytical error of 1 m. Eq. is admitted, the results in this experiment require no further explanation, but serve to reemphasize the effect of slight changes of serum base upon the relationship between base and water balance. In most of the experiments calculation of  $\Delta W$  from base balances alone (Equation 3), without correction for changes in concentration of base in body water, gives results which are much inferior to those obtained by the use of Equation 2 in which this correction has been attempted. The most striking exception to this statement occurs in Experiment 9 in which the average concentration of base throughout the body fluids appears to have

remained fairly constant despite the change in its concentration in serum water. A possible explanation will be offered in the discussion. In 2 instances, Experiment 4 and the second period of Experiment 6, there has been an actual storage of base which, when corrected for changes in concentration by Equation 2, yields strongly negative values for  $\Delta W$  which are in fair agreement with the observed weight changes. Too much stress should not be placed upon the quantitative agreement between the calculated water exchange and observed body weight changes in the first 3 experiments since it will be recalled that the values for serum base are unreliable. These changes are, however, at least in the proper direction to support the validity of our concepts. Because the body weight changes can hardly be taken to represent  $\Delta W$  in Experiment 10, a different method of analysis must be used. Under the conditions of this experiment (v. s.) the tissue wastage could hardly have been greater than 2 kgm. or less than 1 kgm. A body weight gain of 0.35 kgm. was noted. The water gain, then, must have been no more than 2.35 kgm., nor less than 1.35 kgm., which would require the retention of between  $2.35 \times 140 = 330$  m. Eq. and  $1.35 \times 140 = 189$  m. Eq. of Na + K. Actually 546 m. Eq. were retained, leaving between 216 and 357 m. Eq. to



increase the concentration of base in approximately 47 kgm. of body water by between 4.6 and 7.6 m. Eq. per liter. The observed increase of base in the water of the serum was 5 m. Eq. per liter, thus supporting the contention that changes in the concentration of base in the water of the serum are reflected throughout the body water and that corrections for such changes must be made in calculating water exchange from base balances.

Calculations of extracellular fluid exchange ( $\Delta E$ ) from Na by Equation 4 or from Cl by Equation 5 show remarkably good agreement in most instances. The large discrepancies noted in Experiments 2 and 3 are not to be taken too seriously because of the known analytical errors in these experiments. In several of the experiments the changes in the concentration of Na and Cl in the serum exert very appreciable effects on the calculation of  $\Delta E$ . For example, in the second period of Experiment 6, both Na and Cl balances were positive during a profuse diuresis. During the diuresis, however, the concentrations of these ions in the water of the serum increased more than could be accounted for by the amounts retained. Consequently the calculated values for  $\Delta E$  are both slightly negative. In the first period of Experiment 5 the observed losses of Na and Cl were not proportional to their concentrations in the body fluids. Yet the observed changes of concentrations of these electrolytes in the serum were of such magnitude and direction that the values for  $\Delta E$  calculated from both electrolytes are almost identical. In the first period of Experiment 8, the Cl balance was positive while the Na balance was negative. The observed changes in concentration are such, however, that calculation of  $\Delta E$  from either Na or Cl indicates a slight increase in the volume of extracellular fluids.

#### DISCUSSION

That the proposed formula (Equation 2) for the calculation of the total water exchange of the body is fundamentally sound seems probable from the results of these experiments. In most of the experiments the values derived from this formula are in satisfactory agreement with the observed changes of body weight. The exchanges so calculated are generally far superior to those calculated from base balances alone (Equation 3).

This implies the essential correctness of the assumptions made in the derivation of Equation 2, namely, that changes of the concentration of base in the water of serum are equalized throughout the water of the body and that this water makes up approximately 70 per cent of the body weight. The first assumption is true, of course, only if osmotic equilibrium between the various portions of the body fluids is established at the times when periods are begun and ended. This was probably not true in Experiment 7 which may explain the poor results obtained by Equation 2 in this case.

In this experiment, the subject of which had diabetes insipidus, calculation of water exchanges gives absurdly high values. That the water of the body in this instance may have been considerably less than 70 per cent of the body weight is admitted. With the extreme changes of concentration noted in this experiment, the effect of error in this assumption on the calculations may be great. If instead of 70 per cent of the body weight, 50 per cent is used, however, the calculated exchanges will still be unreasonably high. It is possible to speculate upon the mode of production of osmotic gradients of the proper direction to reconcile the findings. The tremendous volumes and low salt concentrations of the urine of the diuretic periods are indicated in Table V. The excretion of such large amounts of water without appreciable amounts of salt should cause a rise in the concentration of base in the serum unless the water is constantly replenished by absorption from the gastro-intestinal tract. If fluid intake is now restricted so that alimentary absorption is eliminated, continued diuresis should result in a rise in the base of the serum so rapid that large gradients may be established between serum and tissues. Water was taken in large amounts during the diuretic periods of this experiment except for a few hours before the periods were terminated. Regardless of the changes of concentration of base noted for the periods as a whole, then, it seems reasonable to infer that at their conclusion a rapid increase in the concentration of base in the serum took place, in which event a gradient of 3 m. Eq. between the water of serum and tissues is not improbable. Values for the base of body fluids as a whole, assuming the above gradients, are given in column 5 of Table V. Because the conditions at the start of

TABLE V  
Additional data and calculations from Experiment 7

Period number	1.	2.	3.	4.	5.	6.	7.	8.
	Urine			Concentration of base in		$\Delta W$ from Equation 2 $\Delta B$ derived from column		Change of weight of body
	Volume	Na	Cl	H <sub>2</sub> O of serum (determined)	H <sub>2</sub> O of body (assumed average)	4	5	
	liters per day	m. Eq. per liter	m. Eq. per liter	m. Eq.	m. Eq.	kgm.	kgm.	kgm.
I...	5.15	9	10	163		+3.48		+1.08
II...	1.03	41	43	153	150	+3.69	+2.60	+1.76
III...	5.39	12	18	143	143	-4.13	-3.14	-2.15
IV...	7.74	17	19	154	151	-0.06	-0.06	+0.10
V...	1.57	96	81	157	154	+5.18	+4.11	+3.19
				146	146			

Period 1 are not definitely known, no attempt has been made to assume a gradient at that time. No water was taken during the whole night previous to the conclusion of the pituitrin periods and diuresis had not set in, so that no gradient need be assumed at these times. Total water exchanges recalculated by Equation 2, using instead of the observed values for base in the water of serum the assumed values for base in body water as a whole, are presented in Table V, column 7. In each period the results so obtained approach more nearly the observed changes of weight. While this discussion is highly speculative it calls attention to the possibility of the existence of osmotic gradients within the body which may require consideration under certain conditions. The particular gradients assumed in this instance have no quantitative significance and are of directional significance only in so far as they rest on reasonable, though yet unproved, inferences concerning the physiological disturbance in diabetes insipidus. Experiments to demonstrate the actual nature of gradients under these conditions are contemplated. Attempts to locate the point of maximum gradient between the various body fluids must likewise be highly speculative. If tissue cells behave like red blood corpuscles, however, their adjustment to the immediately contiguous portions of the body fluids must be almost immediate. Likewise changes in the osmotic pressure of the serum must be transmitted very rapidly to the portions of the extracellular fluids immediately adjacent to the capillaries. Redistribution of base throughout the many ramifications of the extracellular fluids,

however, can occur only through diffusion which is presumably a relatively slow process so that the major portion of the gradient probably lies in the extracellular fluids. To cite an extreme example, a large effusion which has contact with the capillaries only over the pleural surfaces would be expected to adjust itself completely to changes in serum concentration very slowly.

That the water exchange calculated by Equation 3 is superior to that calculated by Equation 2 in Experiment 9 has already been noted. There is a gain of about 3.75 kgm. of water, which in itself should require the retention of  $3.75 \times 142 = 532$  m. Eq. of Na + K. Actually only 518 m. Eq. were retained, leaving no excess to increase the concentration of base throughout the body fluids to the same extent as it was observed to increase in the water of the serum. That is, either the osmotic pressure of the serum was higher than that of the cells at the end of the experiment or the osmotic pressure of the cells was increased by something other than accession of base. The alternative in this instance may lie in a change in the reaction of the body fluids. At the start of the experiment the CO<sub>2</sub> content of the serum was 86.9 volumes per cent as a result of previous depletion of Cl through vomiting. At the conclusion of the experiment this had returned to the normal level of 65.3 volumes per cent. The shift in reaction in an acid direction which presumably accompanied the fall of CO<sub>2</sub> would cause the cells to swell if the osmotic pressure of the surrounding interstitial fluid remained constant. It is possible that the rise in osmotic



pressure of this fluid indicated by the observed rise of serum base was equalled in the cells without change of concentration of base by a diminution of the amount of base bound to protein which because of its large base-combining power is relatively ineffective osmotically. Changes of this nature are known to occur in red blood corpuscles when the reaction of the blood is changed.

That the values for extracellular water exchange calculated by Equations 4 and 5 are in good agreement in most cases has been noted. This lends support to the assumptions made in the derivation of these equations, namely that Na and Cl are distributed similarly through a restricted portion of the body fluids, probably the extracellular portion, that changes of the average concentrations of these ions in these fluids parallel those in the water of serum, and that the extracellular fluids comprise something of the order of 20 per cent of the body weight.

The volume of water in the cells is determined not only by the K balance but also by the total concentration of base in their environment, the interstitial fluids.<sup>4</sup> If the concentration of base does not change, intracellular water exchange and K balance should parallel one another; or if the amount of K in the body remains constant, cell water must vary roughly in inverse proportion to the concentration of base in the water of serum; or if both the K content of the body and the base concentration in its water vary, each of these changes will affect the intracellular water content, and their effects may lie in the same or opposite directions. Thus in Experiment 8, in spite of the fact that K was retained during both periods, the changes of base concentration were such that a marked gain of cell water must have occurred during the first period and a marked loss during the second period. This leads to a consideration of the K exchanges of the body. Transfer of base across the membrane of the human red blood cell has never been demonstrated either *in vitro* or *in vivo*. It seems reasonable to believe that the

red cell does not change its base content during its life in the circulation, in which case it must make its osmotic adjustments chiefly by changes in water content. As a consequence it is possible to cause hemolysis of these cells with ease *in vitro* by dilution of the blood with water or solutions of diffusible substances and with difficulty *in vivo* by extreme changes in osmotic pressure such as those occurring in severe water intoxication (40). The impermeability of other body cells must be facultative, however, as evidenced by changes in the K content of the body observed in balance studies. When marked storage or wastage of protein occurs, similar changes in K are usually noted. Large exchanges of K have been noted, however, in instances when the body is presumably in N equilibrium, for example the K loss noted in the diuresis produced by the administration of acidifying salts (41). Furthermore, K loss out of proportion to the loss of protein has been noted in diabetic acidosis (42). In both of these instances the excretion of K helps to combat excessive change in the reaction of the body by supplying base for the excretion of abnormal accumulations of acids. This loss of cellular base also serves to prevent excessive swelling of the tissue cells which might result from the change of reaction of the tissues and in the latter case, from decrease of concentrations of extracellular base as well. Such excessive swelling might be detrimental not only from the standpoint of cellular function but also because it would serve to deplete further the already diminished volume of extracellular fluids.

The  $\frac{\text{potassium}}{\text{nitrogen}}$  ratio of the urine of Gamble's fasting children exceeded the same ratio in muscle water, as calculated from the analyses of Katz (4). Gamble assumed that, for each gram of protein metabolized, a definite fixed amount of cellular water together with its potassium was freed. Potassium excreted in excess of that estimated in this manner to result from the destruction of protoplasm he attributed to diminution of cell water. He suggested that this diminution might be the result of glycogen depletion. It has been shown above, however, that cellular water may vary over a wide range without any change of the nitrogen or glycogen or even of K balance as the result of a change in the osmolar

<sup>4</sup> The distribution of water between cells and serum is presumably affected also by changes in the pH of the fluid media of the body. There is no knowledge of the quantitative aspects of this change. In only one of the present experiments, which has already been discussed, need this be considered, since in the others there were probably no significant changes of pH.

concentration of the body water as a whole through the addition or removal of water without base or of a change in the concentration of sodium in interstitial fluids, which necessitates an exchange of water with the cells in order to restore osmotic equilibrium. The treatment accorded the data of these experiments presents compelling evidence in support of the objections previously raised (43) to the concept that cellular protein or glycogen is associated in such fixed proportion with water that the excretion of one inevitably entails the elimination of an equivalent amount of the other.

Changes in the calculated volume of extracellular and intracellular fluids may occur independently of one another and may even be in opposite directions. Thus, in the second period of Experiment 5, when the salt stores of the body have been restored to a greater extent than the water stores of the body, as indicated by the observed increase in the concentration of base in body water, the calculated gain of extracellular fluid exceeds the total gain of water by the body. That is the volume of interstitial fluid has gained not only from exogenous sources but also at the expense of the cells, which have yielded water in the reestablishment of osmotic equilibrium. In Experiment 6, when pituitrin was withdrawn, in the second period, a profuse diuresis resulted during which 36 m. Eq. of Na were retained and the concentration of sodium in the water of serum increased 8 m. Eq. per liter. Calculation of water exchanges indicates that the entire diuresis has occurred at the expense of the cells, which, by yielding water, raise their osmotic pressure, without accession of K, to the new level established in the serum. During the first period of Experiment 8 there was a gain of 1.60 kgm. in weight and a calculated gain of 1.83 kgm. of water without significant change in the Na and Cl balance of the body, and with a marked decrease in the concentrations of these ions in the serum. Calculation of  $\Delta E$  by Equations 4 and 5 yields values of 0.17 and 0.45 kgm. respectively. That is, the major portion of the water gain occurred in the cells which, by swelling, had adjusted their osmotic pressure to the lowered level established in the interstitial fluids. In the second period, water balance was again positive but sufficient Na and Cl were retained to supply all the retained fluid, and in

addition to increase the concentrations of these ions in the interstitial fluids. Apparently the base concentration in the cells was increased by the surrender of water to the interstitial fluids, the volume of which, therefore, increased from both exogenous and endogenous sources. The calculated values for  $\Delta E$  are 3.86 and 4.22 kgm. respectively by Equations 4 and 5 while  $\Delta W$  calculated from Equation 2 is only 2.60 kgm. and body weight gain was only 3.31 kgm.

Changes of the concentrations of serum proteins in the shorter experiments probably occurred only through changes of serum volume and can consequently serve as a measure of the latter. This is not true, of course, in conditions such as mercury poisoning, in which large amounts of protein may be lost from the circulation. The exchange between serum and the remainder of the interstitial fluids is not directly affected by electrolyte changes since the capillaries are freely permeable to electrolytes. Therefore changes in serum volume and the volume of interstitial fluids need not parallel one another. In Experiment 9 a large gain in extracellular fluid volume was accompanied by a proportional fall of serum protein. However, large gains of extracellular fluid volume occurred in the second periods of Experiments 5 and 8 without any significant changes of serum protein, and, therefore, presumably of serum volume. When pituitrin was given, the blood volume increased markedly in these experiments. In Experiment 6, the serum proteins fell from 8.50 to 7.50 per cent when pituitrin was given, indicating an increase of approximately 13 per cent in serum volume. During the same time the volume of extracellular fluids increased only  $0.34/50 = 7$  per cent of the original volume. A much more striking disparity occurred in the second period of the same experiment when the serum volume fell markedly without any significant change in extracellular fluid volume as calculated from the metabolism of either Na or Cl.

#### SUMMARY AND CONCLUSIONS

Formulae have been derived for the calculation of total water exchange and extracellular water exchange from changes in the electrolytes of the body. These formulae have been tested by application to experimental studies in man.

Exact quantitative accuracy is not claimed for

the methods of calculation of water exchange presented in this paper. They serve, however, to throw some light upon the mechanisms of fluid exchange between the various compartments of the body fluids as well as between the body and the environment.

The results of the experiments, moreover, tend to confirm certain assumptions which have been made concerning the distribution of the bases and chloride in the body. These are:

1. That the concentration of total base throughout all of the water of the body is approximately alike and that change of the concentration in any portion of the water is equalled in all other portions.

2. That Na is almost entirely confined to the extracellular portions of the water of the human body and that the same is true of Cl with the exception of the small amount of Cl present in the red blood corpuscles.

3. That the total water of the human body comprises approximately 70 per cent, and the extracellular portion 20 per cent, of the body weight.

That extracellular and intracellular fluid volumes may vary independently of each other and of blood volume has been indicated.

It has been suggested that in certain exceptional conditions gradients of osmotic pressure may be set up between serum and tissues and that changes of reaction of the body may distort the normal relation of base to water in the cells and interstitial fluids.

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# THE CHANGES IN THE DISTRIBUTION OF BODY WATER ACCOMPANYING INCREASE AND DECREASE IN EXTRACELLULAR ELECTROLYTE

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Considerable attention has been given the relation of body water to the metabolism of fixed base. Gamble, Ross and Tisdall (1) have pointed out that loss or gain of sodium is usually accompanied by an increase or decrease in extracellular water sufficient to keep the extracellular sodium constant in concentration. Furthermore, since nearly all of the potassium of the body is intracellular and variations in the concentration of potassium in serum are relatively small, a loss or gain in potassium is usually accompanied by an increase or decrease in cellular water sufficient to keep the osmotic pressure of cellular fluid in equilibrium with that of blood plasma. These authors have estimated the probable loss of water from intracellular and extracellular spaces which accompanies the losses of sodium and potassium during starvation. Gamble, Blackfan and Hamilton (2) have described the probable mechanism of the diuretic effect of acidifying salts by similar reasoning. Analogous studies have been made in diabetic coma (3) and infantile diarrhea (4). Since such studies are based on the premise of a parallel loss of water and electrolyte, they do not take into account the redistribution of body water which must take place when electrolyte is lost or gained without a corresponding loss or gain in water. The present study describes the changes in concentration of water and electrolyte in serum and erythrocytes when the amount of extracellular electrolytes is altered with little change in total body water. The probable shifts of body water which explain these changes are discussed.

The experimental procedure used to produce variation in extracellular electrolyte without significant alteration in total body water is based on experiments described by Schechter, Cary, Carpenteri and Darrow (5). These experiments demonstrated that when a watery solution was placed in the peritoneal cavity, it tended to assume the composition of a fluid in ionic and osmotic

equilibrium with blood plasma. Thus when "isotonic glucose solution" (5 per cent) was placed in the peritoneal cavity, sodium, chloride, and bicarbonate diffused into the fluid while glucose diffused into the blood. At first the rate of diffusion of electrolyte into the peritoneal fluid was so rapid that water also passed into the peritoneal cavity. However, after four to six hours the amount of fluid in the peritoneal cavity was approximately that injected, but the composition approached that of a fluid in equilibrium with plasma. In guinea pigs about twenty-four hours was required for complete absorption; the interval in dogs, monkeys and rabbits was not determined, but is probably between twelve and twenty-four hours. Since the fluid in the peritoneal cavity was not immediately available throughout the tissues, the body may be considered to have lost temporarily the amount of electrolyte present in the peritoneal cavity, while the total amount of body water remained relatively unaltered. The interpretation of the results is simplified by the fact that anuria accompanies these experiments and the only loss of water is by evaporation and the only gain, from cellular oxidations, which for the present purposes could be neglected.

In order to increase the amount of extracellular electrolyte, saline of double physiological strength (1.8 per cent NaCl) was injected into the peritoneal cavity. In these experiments after about four hours, the fluid in the peritoneal cavity was in approximate equilibrium with the blood plasma and the total volume was approximately that injected. In this case the total amount of extracellular electrolyte was increased with relatively small change in total body water.

## EXPERIMENTAL PROCEDURE

Healthy dogs, rabbits and monkeys (*Macacus rhesus*), maintained on an adequate diet, were used as the experimental animals. The last feeding occurred about eighteen hours before the experimental procedure. Food and water were removed from the cages during the course of the experiments.

Blood specimens (about 30 cc. in dogs and 20 cc. in rabbits and monkeys) were taken from the external jugular vein in dogs, directly from the heart in rabbits and from the femoral vein in monkeys. About 10 cc. of the blood were immediately transferred to a tube containing a small amount of mercury and securely stoppered. The size of the tube and the amount of mercury used were such that the blood completely filled the tube. Defibrination was accomplished by gentle inversion of the stoppered tube for about five minutes. The remainder of the blood was delivered under mineral oil, allowed to clot at room temperature, centrifuged and the clear serum removed.

Soon after withdrawal of the first specimen of blood, a five per cent solution of glucose was injected into the peritoneal cavity in one series of animals. The glucose solution was freshly prepared and sterile, and the amounts injected were approximately 100 cc. per kgm. of body weight in most of the experiments. The second series of animals received similar quantities of freshly prepared, sterile 1.8 per cent solution of sodium chloride intraperitoneally. In both cases the solutions were warmed to body temperature before injection and gave no evidence of being irritating. In the experiments on dogs the urine was collected in cages equipped for metabolic studies. In the experiments on rabbits and monkeys clean dry pans were placed under the wire cages. About four to six hours later, a second specimen of blood was obtained and treated in the same way as the first specimen. Samples of the peritoneal fluid were obtained at this time in the dogs, but in the monkeys and rabbits it was usually impossible to obtain peritoneal fluid without killing the animals and this was deemed unnecessary in most of the experiments.

In order to determine the amount of fluid remaining in the peritoneal cavity after approximate equilibrium had been established, fourteen animals were sacrificed and the peritoneal fluid drained and measured. These animals were also carefully examined for gross pathological changes. In four of the animals a measure of the total volume of material in the gastro-intestinal tract was made. No histological studies were carried out.

#### CHEMICAL METHODS

The volume of the blood cells was determined on the defibrinated blood by centrifuging at high speed for 20 minutes in capillary tubes. Measurements of the heights of the columns of blood and cells were made with calipers. The following chemical determinations were made: protein, by macro-Kjeldahl (6) method using superoxol to insure complete digestion; sodium, by Butler and Tuthill's modification of the method of Barber and Kolthoff (7), without removal of phosphate on serum but with removal of phosphate on whole blood; chloride, by Patterson's (8) method, using 0.5 cc. samples; water, by weighing before and after drying overnight at a temperature of 105° C. All determinations were made in duplicate. The concentrations in the cells were calculated from the hematocrit values and the con-

centrations in whole blood and serum by the following formula:  $C = \frac{B - S(1 - V)}{V}$ , where  $C$  represents cell concentration;  $B$ , whole blood concentration;  $S$ , serum concentration and  $V$ , the proportion of red cells in whole blood.

#### EXPERIMENTAL RESULTS

During the course of the experiments, data were collected which permitted estimation of the changes in concentration of electrolyte and water in serum and erythrocytes. The present paper presents such data as bear on the probable changes in distribution of water produced by the experiments. In a second paper (14) the changes in the concentration of electrolytes and water in erythrocytes will be discussed. Mention of the latter findings will be made in the present paper only inasmuch as the changes found in this accessible cell are used to explain the clinical symptoms of the animals and the probable changes in distribution of water in the body.

#### A. Amount of fluid remaining in the peritoneal cavity

Table I shows the amount of fluid recovered at autopsy after a lapse of time comparable to that

TABLE I  
Volume of fluid remaining in the peritoneal cavity

Animal	Experiment number	Weight	Solution injected	Amount injected	Amount recovered	Time elapsed	Urine
		kgm.		cc.	cc.	hours	cc.
Dog 1...	1	6.4	Glucose	500	467	6.0	5
Dog 6...	16	6.6	Glucose	500	587	5.0	8
Dog 3...	7	6.1	Glucose	550	570	4.5	0
Dog 7...	17	11.4	Glucose	500	542	6.0	0
Monkey 2	8	2.8	Glucose	250	228	3.7	0
Monkey 3	9	6.5	Glucose	430	400	3.0	0
Rabbit 3.	18	2.5	Glucose	250	260	4.0	30
Rabbit 4.	14	2.0	Glucose	200	186	4.0	0
Rabbit 5.	15	3.0	Glucose	275	240	4.0	0
Rabbit 9.	16	2.6	NaCl	260	250	4.2	8
Rabbit 10	17	2.6	NaCl	260	225	4.2	5
Rabbit 11	18	3.8	NaCl	370	415	4.0	5
Rabbit 12	19	2.9	NaCl	280	255	4.5	0
Rabbit 13	20	3.4	NaCl	330	310	4.0	5

used in the experiments. The data show that four to six hours after the injection of either a five per cent solution of glucose or a 1.8 per cent solution of sodium chloride into the peritoneal cavity, the amount of fluid remaining is approximately that injected. In both the "glucose" and "saline" experiments the changes in the total amount of peritoneal fluid amounted to an increase or decrease of less than ten per cent of

the amount injected. Since the amount of fluid injected was ten per cent of the body weight and since total body water is about seventy per cent of the body weight, the experiments would change available body water by less than 1.5 per cent. Because blood plasma, interstitial fluid and intracellular fluid are all in osmotic equilibrium, the effects of this change in body water will be distributed throughout all body fluids and be of minor importance in explaining changes found in the blood. In other words the chief changes produced in "the glucose" experiments are brought about by withdrawal of extracellular electrolyte and the chief changes of the "saline" experiments are the result of the increase in extracellular electrolyte (NaCl).

The preponderant effect of loss or gain of sodium and chloride becomes apparent when one considers the losses or gains of these ions in relation to their total amounts in normal animals. The approximate losses of chloride and sodium in the "glucose" experiments may be estimated by multiplying the original volumes (or the final volume when this was determined) by the final concentrations of these ions in the peritoneal fluid. These calculated losses of chloride and sodium have been compared to the total amounts of chloride and sodium found in a normal dog, a normal monkey and two normal rabbits (unpublished data from this laboratory). In the "glucose" experiments the losses of chloride are about 25 per cent of total body chloride; the losses of sodium, about 20 per cent of total body sodium. In the "saline" experiments the effective increases in body sodium and chloride are more uncertain, especially in those animals excreting considerable quantities of urine. Neglecting excretion in the urine which was negligible in most of the experiments, similar calculations show that the increases in body chloride were about 50 per cent of total body chloride and the increases in sodium, about 30 per cent.

It was not deemed necessary to sacrifice dogs and monkeys in order to determine the amount of fluid remaining in the peritoneal cavity after the injection of 1.8 per solution of sodium chloride since the behavior of the various animals was similar and the appearance of the abdomen and the ease with which fluid could be withdrawn from the peritoneal cavity was the same in the "saline" and "glucose" experiments.

### *B. Clinical aspects of animals subjected to removal of extracellular electrolyte with little alteration in total body water*

Following the intraperitoneal injection of glucose solutions, the animals suffered no local signs of pain or irritation. However, in all animals (8 dogs, 5 rabbits and 5 monkeys) signs of mild to severe dehydration developed which were comparable to those observed in dehydrated patients. The tongue and mucous membranes were dry; the skin showed loss of turgor; the animals became languid and looked sick. In the monkeys and rabbits greyish pallor such as is seen in "alimentary intoxication" could be distinguished. In about 24 hours the animals recovered completely.

Following the injection of isotonic glucose, no urine was passed by any of the animals during the periods of experimental observation which were four to six hours. In view of the clinical evidences of dehydration it was interesting to note that the animals were not thirsty. Autopsies on the animals listed in Table I confirmed the dryness of the mucous membranes, for only 5 cc. of mucoid material could be recovered from the stomach and small intestines in the dogs and monkeys. The small intestines of the rabbits contained little fluid but, as is the rule in this species, the stomach and colon were filled with considerable material which, however, was not very moist.

### *C. Changes in the blood in the "glucose" experiments*

Data which concern the distribution of water in serum and erythrocytes are given for representative experiments in Table II. As was pointed out previously, the various data may be regarded as exemplifying the changes produced by the losses of extracellular electrolyte with little alteration in body water.

In all experiments the proportions of red cells in blood and the concentrations of serum proteins were increased. The increases in the proportion of red cells were considerable. If one take no account of the blood withdrawn for analyses, one may calculate the apparent loss of plasma which would produce the given change in the proportion of erythrocytes. Because loss of blood tends to be replaced by interstitial fluid, the calculation probably underestimates the diminution of plasma



TABLE II

*Changes in the blood following intraperitoneal injection of 5 per cent glucose*

Animal	Experiment number	Weight	Fluid injected	Time elapsed	Cell volume	Serum protein	Serum water	Serum Na	Serum Cl	Cell water	Cell protein	Peritoneal Na	Peritoneal Cl
		kgm.	cc.	hours	per cent	per cent	per cent	m. Eq. per L.	m. Eq. per L.	per cent	per cent	m. Eq. per L.	m. Eq. per L.
Dog 3.....	4	6.0	500	0.0 4.5	39.6 52.2	4.8 5.8		143.3 130.7	112.4 92.6		33.6 28.9	114.3	96.2
Dog 1.....	1	6.4	500	0.0 6.0	48.7 57.2	6.4 8.4		147.3 132.5	106.1 86.8		32.8 30.3	115.0	91.1
Dog 3.....	5	5.5	500	0.0 4.5	34.0 44.8	5.7 6.9		144.1 124.4	112.6 91.8		31.9 29.1	107.8	91.8
Dog 3.....	7	6.1	550	0.0 4.0	44.9 54.4	5.8 8.0	92.4 90.3	147.5 131.7	111.1 91.2	74.4 75.4	30.3 28.6	105.3	88.2
Monkey 4..	10	3.8	400	0.0 4.5	43.7 55.2	6.8 8.0	90.9 88.9	144.6 125.2	104.5 82.9	76.7 78.8	29.0 25.9		
Monkey 4..	11	3.5	300	0.0 4.0	41.8 46.8	7.7 8.3	90.6 89.8	150.2 131.4	108.5 90.4	75.9 76.5	30.8 28.2	125.8	99.4
Monkey 2..	8	2.8	250	0.0 3.5	47.0 58.5	7.4 9.5	90.8 88.2	165.9 145.3	109.3 96.2	75.4 76.5	29.7 28.9	92.9	75.6
Monkey 3..	9	6.5	430	0.0 4.0	50.0 55.0	7.5 8.6	90.6 89.7	154.5 133.5	110.1 92.3	76.4 76.5	28.5 28.9	125.1	98.8
Rabbit 4...	14	2.0	200	0.0 4.0	35.5 38.3	6.1 6.8	92.5 91.8	137.5 119.3	95.4 85.3	76.3 79.4	29.6 25.9	98.4	78.7

volume which would otherwise have been produced by the loss of extracellular electrolytes. Roughly, by this calculation, the losses of plasma volume varied from 8 to 27 per cent of the total blood volume, or 18 to 49 per cent of the plasma volume. Similar calculations of the apparent losses of plasma water from the changes in concentration of serum proteins gave values roughly agreeing with those calculated from the changes in the proportion of red cells. Although changes in plasma volume are not the only factors affecting the concentration of cells in blood or proteins in serum, in experiments of short duration, changes of the magnitude shown in Table II could hardly occur without being due chiefly to losses of water from plasma.

The water of red cells increased as is evidenced by reductions in the concentration of cell proteins. Reductions in concentration of cell proteins occurred in all experiments except in the case of Monkey 3, Experiment 9, when the changes were so slight as to be within the error of the methods. The increases in erythrocytic water amounted to 1.4 to 7.3 per cent of the original cellular water.

Since total body water changed relatively little, this water was presumably derived from extracellular spaces.

In all cases considerable reductions were found in the concentrations of chloride and sodium in the serum. It is obvious that these reductions were chiefly brought about by migration of sodium and chloride into the peritoneal cavity.

*D. Clinical aspects of animals subjected to increase of extracellular electrolyte with little alteration of body water*

The intraperitoneal injection of 1.8 per cent solution of sodium chloride produced few clinical symptoms. The animals retained their appetites and did not look ill although two dogs vomited. They became thirsty but no loss of skin turgor occurred. Grossly the rabbits which were examined postmortem revealed nothing abnormal except the fluid in the peritoneal cavity. No ill effects were noted after 24 hours.

In three of the experiments on dogs a definite diuresis occurred, resulting in the loss of about 25 per cent of the fluid injected (see Table III). In



TABLE III  
*Changes in the blood following intraperitoneal injection of 1.8 per cent NaCl*

Animal	Experi- ment num- ber	Weight	Fluid in- jected	Time elapsed	Cell volume	Serum protein	Serum water	Serum Na	Serum Cl	Cell water	Cell protein	Peritoneal		Urine		
												Na	Cl	Vol- ume	Na	Cl
		kgm.	cc.	hours	per cent	per cent	per cent	m. Eq. per L.	m. Eq. per L.	per cent	per cent	m. Eq. per L.	m. Eq. per L.	cc.	m. Eq. per L.	m. Eq. per L.
Dog 3.....	4	6.5	600	0.0 4.5	45.1 38.5	6.7 6.1		140.1 157.6	108.2 132.0		31.6 32.6	165.1	160.0	0	—	—
Dog 3.....	1	5.3	500	0.0 4.5	38.5 35.0	6.0 5.6		146.2 162.7	110.8 131.4		32.7 34.2	168.8	153.0	0	—	—
Dog 4.....	5	11.6	1000	0.0 5.0	64.4 56.1	5.9 5.9		142.0 152.9	111.0 135.0		31.8 32.9		156.0	360	218	206
Dog 4.....	3	11.2	1000	0.0 4.0	37.5 34.8	6.2 6.3		147.1 161.4	112.2 130.6		34.1 34.2			240	—	380
Dog 5.....	8	7.0	700	0.0 4.0	48.1 45.3	6.2 6.2	91.9 92.1	145.8 161.1	111.6 129.8	74.3 74.2	31.7 32.7	166.6	150.1	180	319	339
Monkey 4.	10	3.7	350	0.0 4.0	36.0 27.4	7.4 6.1	90.7 91.9	148.2 165.1	107.4 130.7	76.9 74.5	27.2 29.4			±*		
Monkey 3.	9	5.8	425	0.0 5.0	46.2 40.2	7.6 7.3	90.6 91.1	147.2 163.9	104.9 125.0	76.2 74.0	29.4 31.9	164.7	140.9	±		
Monkey 5.	11	2.6	260	0.0 4.5	41.9 35.0	7.5 6.5	91.1 91.9	144.9 169.4	107.5 142.1	75.0 73.4	32.5 33.6			±		
Monkey 5.	12	2.5	225	0.0 3.8	38.8 29.3	6.9 6.1	91.3 91.4	147.0 165.3	107.4 132.2	75.3 74.5	31.7 34.8			±		
Rabbit 7..	14	2.5	250	0.0 4.0	30.8 26.5	4.9 4.4	93.7 94.3	138.7 152.0	103.3 121.8	74.7 73.3	31.9 32.6			±		
Rabbit 8..	15	1.9	180	0.0 4.0	40.4 27.0	7.7 6.2	90.8 92.2	152.8 175.6	123.1 154.0	76.3 74.8	29.9 31.5	182.4	172.1	±		

\*± indicates less than 5 cc. of urine.

these cases the urine contained 200 to 350 milliequivalents of sodium and chloride per liter. In the other experiments practically no urine was passed during the first 4 to 6 hours.

#### *E. Changes in the blood in the "saline" experiments*

The results of the examination of the blood in representative experiments are given in Table III. As was pointed out previously, the various changes may be regarded as exemplifying the alterations produced by an increase in extracellular electrolyte with little alteration in total body water. In both experiments on Dog 3 and in all experiments on rabbits and monkeys, only minimal amounts of urine were excreted. Since in both experiments on Dog 4 and the one on Dog 5, about one fourth of the salt and water injected

into the peritoneal cavity was recovered in the urine, these experiments gave less striking but qualitatively similar changes.

In Dog 3 and in the rabbits and monkeys the concentration of serum protein and the proportion of red cells in blood decreased. The apparent increases in plasma volume were 16 to 52 per cent when calculated from the changes in the proportion of red cells in blood while the apparent increases were 4 to 24 per cent when calculated from the changes in concentration of serum proteins. In each experiment the former calculation led to a greater value than the latter. However, so many factors may influence the concentration of serum proteins or the proportion of red cells in blood that a close correlation is unlikely. The changes are sufficiently great to indicate considerable increases in plasma volume.

The concentrations of proteins in the red cells increased owing to losses of erythrocytic water. The losses of cellular water varied from 2 to 7 per cent of the original water. Because body water remained relatively constant, the water which left the red cells presumably migrated to the extracellular spaces.

In Dogs 4 and 5, the results were much the same as in the other experiments, if consideration be given the large amount of salt and water excreted. The changes in the proportion of red cells indicate considerable increases in plasma volume. However, for some reason which is not apparent, the concentrations of serum proteins did not change. The proteins of the red cells became concentrated owing to losses of cellular water, except in Dog 4, Experiment 8. The changes in serum sodium and chloride in Dogs 4 and 5 are of the same order of magnitude as those of the other experiments.

In all cases considerable increases in the concentration of chloride and sodium in serum occurred owing to migration of these ions from the fluid injected into the peritoneal cavity. The apparent means which the body uses to maintain osmotic equilibrium in both types of experiment will be brought out in the discussion.

#### DISCUSSION

Since the principal phenomena exhibited by the experiments must fit into the concepts of the physiological behavior of water, a tentative description of the factors affecting the distribution of water in the body will be given. Almost seven-tenths of the body is made up of water which moves between intracellular and extracellular spaces in such a manner as to maintain osmotic equilibrium between the fluid of the cells and that in the extracellular spaces. Since about nine-tenths of the osmotic pressure of body fluids is maintained by electrolytes, the distribution of body water is largely determined by the distribution of electrolytes. Furthermore, as the concentration of anions equals the concentration of cations, the approximate osmotic relations are given by the concentrations of the total base in milliequivalents per liter of water. Because the preponderant bases are the univalent ions, sodium and potassium, the concentrations of these cations determines roughly the osmotic pressure. One

may, therefore, surmise that the factors controlling the distribution of sodium and potassium also govern the distribution of water.

From analyses of blood plasma, lymph, edema fluid and cerebrospinal fluid it is fairly well established that all extracellular fluids have approximately the composition of plasma, except that little protein is present in the extracellular fluids outside of the vascular system. The actual amount of extracellular fluid is unknown, but analyses of muscles (9) and whole bodies (10, 11) indicate that about two-tenths of the body weight must be due to extracellular fluid. This estimate is obtained by assuming all but negligible quantities of sodium and chloride are extracellular and that their concentrations in extracellular fluids are essentially those of blood plasma when expressed in concentrations per kilogram of water. Assuming this to be true, about five-tenths of the body weight is made up of intracellular water. The chemical composition of intracellular fluid is not known, but from analyses of red cells (12, 13, 14), muscles (9) and whole bodies (10, 11), one may surmise that the chief anions are proteins and phosphate (chiefly organic) and the chief cation, potassium. This particular distribution of ions with sodium and chloride<sup>1</sup> on one side of the cellular membrane and potassium and phosphate on the other is usually assumed to indicate relative impermeability of cellular membranes to these ions.

The probable changes in distribution of body water which fit in with the concepts briefly outlined above can most readily be brought out with the aid of a diagram (Figure 1). On the ordinate, the concentrations of electrolyte are indicated in milliequivalents per liter of water, while on the abscissa, the volumes of body water are represented in liters. The areas bounded by these lines, therefore, represent the total amount of electrolyte in intracellular and extracellular compartments. The original distribution of body water is given by the continuous lines; the distribution of body water after removal of 100 milliequivalents of extracellular electrolyte is represented by the lines which are made up of dots

<sup>1</sup>The chloride of the erythrocytes is apparently an exception to the rule that intracellular fluids contain little or no chloride.

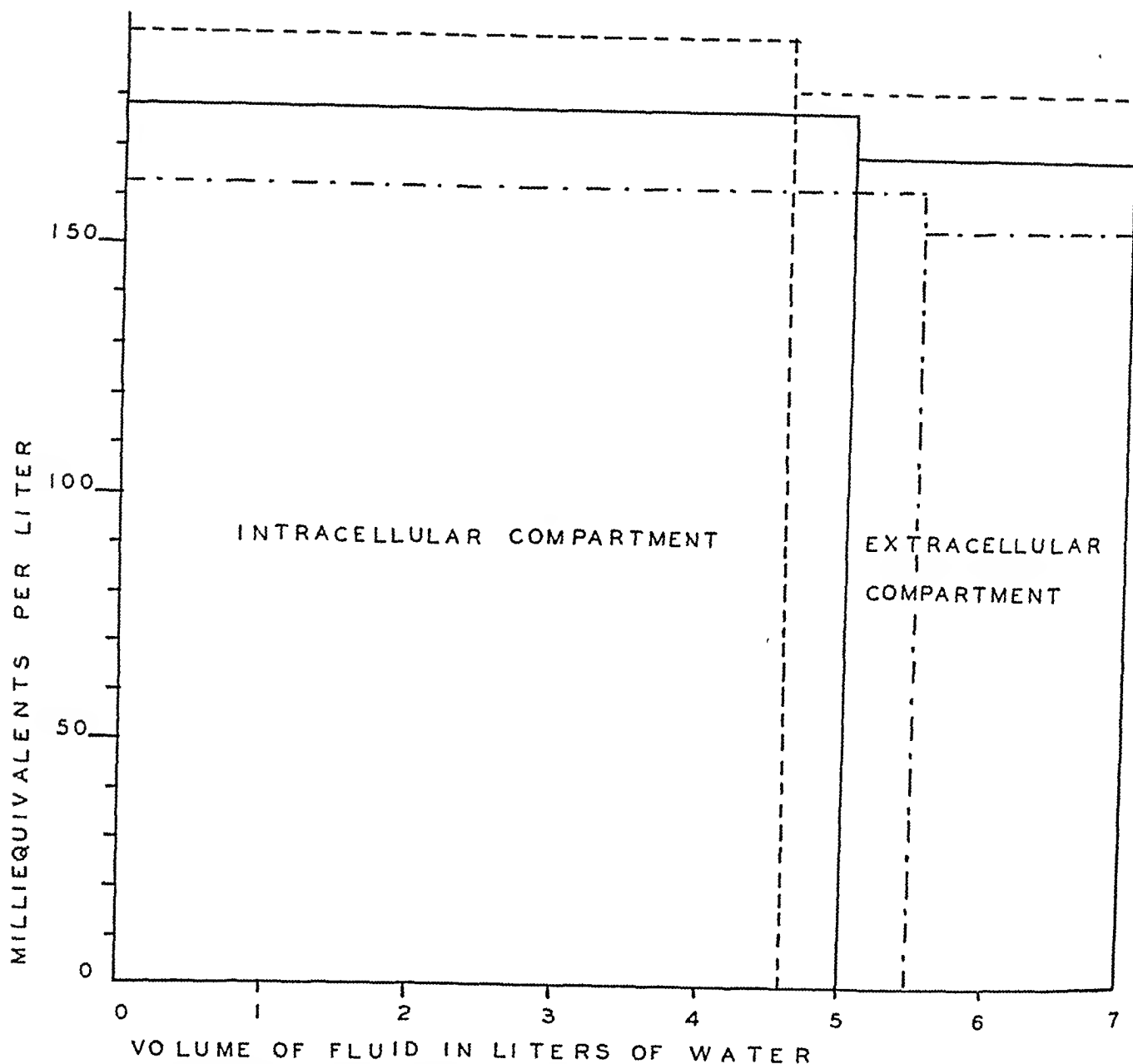


FIG. 1. DIAGRAMMATIC REPRESENTATION OF THE DISTRIBUTION OF BODY WATER AND ELECTROLYTE.

The areas bounded by the lines representing volume and concentration indicate the total amount of intracellular or extracellular electrolyte. The solid lines give the distribution under normal conditions; the lines made up of dots and dashes indicate the distribution after removal of 100 m. Eq. of extracellular electrolyte; the lines made up of dashes represent the distribution after addition of 100 m. Eq. of extracellular electrolyte.

and dashes; the distribution of body water after addition of 100 milliequivalents of extracellular electrolyte is illustrated by the lines made up of dashes. Since by assumption the total amount of intracellular electrolyte does not change, the three areas representing intracellular electrolyte are equal. However, in order to maintain osmotic equilibrium between the intracellular fluid containing a fixed amount of electrolyte and the extracellular fluid containing variable amounts of electrolyte, the volume of intracellular water and

the concentration of intracellular electrolyte must vary as indicated in the diagram. The relation of the changes in amount of extracellular electrolyte to the total body fluid is represented in the diagram in quantities and concentrations comparable to those which the experiments present.

The areas in the diagram representing the shift in water accompanying loss of extracellular electrolyte indicate the probable changes occurring in the "glucose" experiments. This part of the diagram will be discussed first together with the

assumptions on which the diagram is based and the reasons for making these assumptions.

Conceivably osmotic equilibrium between intracellular and extracellular fluids might be brought about by transfer of water across cellular membranes, by transfer of ions across cellular membranes, or by both processes. However, previous work (12, 13) and data obtained in these experiments which will be discussed more fully elsewhere (14) indicate that transfer of water is the chief means of adjusting the osmotic equilibrium between plasma and erythrocytes. Since most cellular membranes are likewise commonly regarded as relatively impermeable to cations, shift of water across cellular membranes is probably the chief means of maintaining osmotic equilibrium between intracellular and extracellular fluids. The diagram represents transfer of water as the sole means of adjusting osmotic relationships.

The experimental observations indicate that shift of water across cellular membranes is probably the chief means of maintaining osmotic equilibrium between body fluids. As was pointed out previously, in the "glucose" experiments the changes in concentration of proteins in serum and red cells in blood indicate apparent losses of plasma volume of 18 to 49 per cent of the original volumes. Loss of skin turgor signifies diminution of extracellular fluids elsewhere than in the blood. The symptoms and signs of dehydration are those which one is accustomed to associate with loss of extracellular fluid. Furthermore, the losses of sodium and chloride are equal to about one fourth of the amounts of these ions in normal animals. If the sodium and chloride remaining in the extracellular fluid had not been concentrated by transfer of water from extracellular to intracellular spaces, the concentrations of serum sodium and chloride would have shown greater reductions than those found. Indication of transfer of extracellular water is given directly by the decreases in concentration of erythrocytic proteins. The "glucose" experiments as a whole fit in with the concepts represented in the diagram and indicate that loss of extracellular electrolyte with little or no change in body water leads to hydration of intracellular fluids and dehydration of extracellular fluids.

In the diagram the loss of extracellular electro-

lyte produces a ten per cent increase in intracellular water and a twenty-five per cent decrease in extracellular fluids. With a given loss of extracellular electrolyte accompanied by a disproportionate loss of water, or, as in the "glucose" experiments by little change in body water, the magnitude of the reduction in concentration of extracellular electrolyte will be dependent, not on the volume of extracellular fluid but rather on total volume of body water.<sup>2</sup> This deduction follows from the fact that the change in osmotic pressure produced by loss of extracellular electrolyte must be equalized throughout all body fluids. Since this adjustment apparently takes place chiefly by shift of water from extracellular to intracellular compartments, the magnitude of this transfer of water will be dependent upon the relation of the volume of extracellular water to total body water. *It is, therefore, apparent that a value for the proportion of body fluid in extracellular spaces is a necessary factor in predicting alterations in the distribution of body water under varying conditions.*

The observations in the "saline" experiments likewise fit in with the concepts expressed in the diagram (Figure 1) showing the changes produced by increase in the amount of extracellular electrolyte without alteration in total body water. In this case, since the increase in extracellular electrolyte augments the osmotic pressure of this fluid, water is attracted from the cells to the extracellular spaces.

In the "saline" experiments, the effective increase in sodium and chloride in the extracellular fluid is uncertain, especially when diuresis occurred. However, in general, the changes in concentration of chloride and sodium are of an order

<sup>2</sup> This relationship for constant total body water and when the only loss of base is loss of Na from extracellular water, may be expressed by the following formula:

$$[Na]_1 V - (Na)_s = [Na]_2 V,$$

where  $[Na]_1$  is the original concentration of sodium in serum water;  $[Na]_2$  is the second concentration of sodium in serum water;  $(Na)_s$  is the change in body sodium and  $V$  is the volume of body water in liters. The application of this formula to the data in the "glucose" experiments yields values for  $V$  (total body water) which fit in with the probable value for total body water, namely, about 70 per cent of total body weight.

of magnitude that indicate that extracellular fluid must have been diluted with water obtained from the cells of the body. The changes in concentration of the proteins in erythrocytes indicate that these cells lost water as is also attested by the decreases in the water of the red cells. Increase in extracellular fluid is difficult to demonstrate because fairly large accumulations of interstitial fluid often give no signs. However, increases in plasma volume are indicated by the decreases in concentration of proteins in serum and of red cells in blood. In other words, the chief finding in the "saline" experiments fit in with the concepts of the diagram and indicate that increase in extracellular electrolyte with little change in body water produces hydration of extracellular fluids and dehydration of intracellular fluids.

It is interesting to note that no urine was excreted during the "glucose" experiments in spite of the reductions in concentration of electrolyte and the probable hydration of the cells. In the "saline" experiments, part of the excess of sodium and chloride in some instances was excreted so as to diminish the disturbances but in two of the experiments on dogs and all of those on rabbits and monkeys, little urine was formed during the period of observation. We have no satisfactory explanation of the reason why the kidneys failed to adjust electrolyte concentration.

Whatever facts about renal adjustment of water volume or electrolyte concentration are discovered, or whatever knowledge concerning permeability of cellular membranes and the actual volumes of the various types of body fluids is developed, the present experiments demonstrate that disturbances in the distribution of body water can occur and induce grave symptoms even when body water is little changed. In acute adrenal insufficiency in dogs (15, 16, 17), a striking loss of sodium unaccompanied by a corresponding diminution of body water has been demonstrated. From a chemical point of view the changes in acute adrenal insufficiency and those produced by loss of extracellular electrolyte in the "glucose" experiments are quite similar. The explanation of the symptoms and chemical evidences of dehydration in adrenal insufficiency is probably also similar to that presented in this paper. After the present experiments were practically completed and the interpretation developed, Gilman (18) re-

peated the essential features of our experiments and showed that a decrease in blood pressure and increase in susceptibility to the effects of hemorrhage occur with loss of extracellular electrolyte and little change in body water. Furthermore disturbances in the distribution as well as the amounts of body water probably occur as the result of disproportionate losses of water and electrolyte in diabetic coma, persistent vomiting, protracted diarrhea, heat stroke, etc. It is also worth noting that the temporary immobilization of extracellular electrolyte following therapeutic injection of solutions of glucose into the peritoneal cavity and subcutaneous tissues will have the same effect as that recorded in the "glucose" experiments.

Gamble, Ross and Tisdall (1) have pointed out that when the loss or gain of base is accompanied by proportionate changes in body water, the changes in the volumes of intracellular and extracellular fluids may be estimated from the concentrations of intracellular potassium and extracellular sodium. However, when the loss or gain of base is not accompanied by a proportionate change in body water such as occurs in pathological disorders accompanied by marked changes in the concentration of sodium in the serum, the estimation of the probable changes in the volumes of intracellular and extracellular fluid cannot be made without a knowledge of the ratio of intracellular potassium to extracellular sodium in the body.

Few reports give data which enable one to calculate the ratio of body potassium to body sodium. Katz (9) found the ratio in adult human muscle to be 2.37; Klose (10) found the ratio in the whole body of an infant to be 0.45 and Iob and Swanson (11), 0.52. In this laboratory (19) the ratio for a dog was 0.84; for a monkey, 1.22 and for two rabbits, 1.41 and 1.44. (The ratios are based on molecular concentrations.) In order to apply ratios derived from analyses of whole bodies, it is necessary to establish how nearly sodium is exclusively extracellular. Furthermore, the probable volume of extracellular fluid must be determined. In any case, it is misleading to speak of intracellular and extracellular water without bearing in mind that *the water of the body may become either intracellular or extracellular, depending on the factors governing its distribution.*

## SUMMARY

Experiments on dogs, rabbits and monkeys are described which induced (1) loss of extracellular electrolyte with little change in total body water and (2) increase in extracellular electrolyte with little change in total body water.

The loss of extracellular electrolyte with little change in body water induced symptoms and signs of dehydration. The concentrations of chloride and sodium in serum were reduced; the concentrations of proteins in serum and the proportions of red cells in blood increased; the concentrations of proteins in erythrocytes were reduced by increases in cellular water. These phenomena are probably referable to shift of extracellular water into body cells producing dehydration of extracellular fluids and hydration of intracellular fluids.

Increase in extracellular electrolyte with little change in total body water induced few symptoms except thirst. The concentrations of chloride and sodium in serum increased; the concentrations of proteins in serum and the proportion of red cells in blood decreased; the concentrations of proteins in erythrocytes increased owing to losses of cellular water. The probable explanation of these findings is shift of water from body cells into extracellular spaces producing dehydration of the cells and hydration of extracellular fluids.

The factors governing the distribution of body water are discussed.

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# THE OCCURRENCE OF ANTIFIBRINOLYTIC PROPERTIES IN THE BLOOD OF PATIENTS WITH ACUTE HEMOLYTIC STREPTOCOCCUS INFECTIONS

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The rapid dissolution of human fibrin clot by *Streptococcus hemolyticus* of the *beta* type is dependent upon the presence in cultures of an enzymic substance which is excreted by the organisms (1, 2). The bacterial product, which induces fibrinolysis, is characteristically elaborated by strains of hemolytic streptococci which cause acute infection in patients. Furthermore, the cultures, obtained from patients suffering with very severe types of illness, have been found to exhibit, with greatest frequency, the most potent fibrinolytic activity (3).

It has also been demonstrated that patients convalescent from acute infection due to hemolytic streptococci often develop a humoral property which is effectively antagonistic to the fibrin dissolving action of cultures of the infecting organisms (4).

Since the fibrinolytic product of streptococci has the properties of an enzyme, the inhibiting action of blood from convalescent individuals manifests itself as a specifically increased antienzymic effect. A review of the literature indicates that attempts to develop antiferments as an immunological process associated with antibody production have given variable results. Consequently it is not possible, at the present time, to state whether the acquired antifibrinolytic properties of patient's blood is dependent upon a specific antibody comparable to other antibacterial immunological processes, or is antienzymic through a special mechanism. Studies are now in progress which are directed toward identifying the antifibrinolytic properties.

In pursuing studies on the occurrence of the antifibrinolytic property of the blood, a number of observations have been made with plasma from a series of cases, both larger in number and more diverse in type than those which comprised the former report. The results are presented in this communication.

The observations have been made for the purpose of obtaining information concerning (1) the frequency with which this special response fol-

lows acute streptococcus infections, (2) the approximate time in the course of the illness at which it was demonstrable, and (3) the relation of the presence or absence of humoral antifibrinolytic activity to the clinical course of the diseases, with respect to either complete recovery, or persistent suppurative complications, or fatal termination.

The patients, who were selected for the study, consisted of forty-five individuals representing different types of acute streptococcus diseases of varying degrees of severity. In some of the cases recovery was prompt and complete; in others, the illness was more severe and was characterized by metastasizing suppurative complications; another group consisted of individuals who died with fatal septicemia. The observations have been further extended to include results obtained with the blood of a few patients with rheumatic fever and acute hemorrhagic nephritis. The list of cases is as follows:

16	cases of erysipelas
5	" " scarlet fever
9	" " acute tonsillitis
3	" " peritonsillar abscess
12	" " severe, progressive suppurative infections, 10 of which had septicemia
8	" " rheumatic fever
5	" " acute hemorrhagic nephritis.

Total 58

The observations were made with samples of blood obtained at repeated intervals during the course of the illness, both before and after recovery. Whenever it was possible, blood was procured at times when changes in the clinical course of the disease occurred, as represented by definite improvement, or the appearance of additional spread of the infection, or an increasing terminal septicemia.

The technical procedures, by which the dissolving action of streptococci on human fibrin is demonstrable, have been previously described in

TABLE I  
Cases of erysipelas

Case number	Patient. History number. Date of admission	Sex and age	Day of disease on admission	Approximate day of recovery	Fibrinolytic test	
					Day of disease (bleeding)	Result
1	Tin. 19028 March 1, 1934	years M. 48	3d	12th	3d 10th 22d	— ++++ ++++
2	Che. 21873 April 22, 1934	F. 31	3d	6th	3d 8th 15th	+ ++++ ++++
3	Mye. 40590 May 9, 1934	M. 25	6th	11th	6th 9th 14th	— + ++++
4	Hol. 53585 February 3, 1934	F. 4	8th	10th	8th 16th	++++ ++++
5	Hop. 52913 December 27, 1933	M. 38	6th	8-10th	6th 14th	+++ ++++
6	Fis. 52966 December 30, 1933	M. 13	9th	9th	9th 16th	+++ ++++
7	Dob. 863 November 30, 1933	F. 26	10th	17th	10th 18th 26th	+ +++ —
8	Ber. 54474 March 25, 1934	F. 38	2d	5th	2d 6th 11th 14th	— — — —
9	Bau. 43418 December 15, 1933	F. 22	2d	9th	3d 8th 12th	+ ++ ++++
10	Car. 53443 January 25, 1934	M. 60	6th	11-18th	6th 12th 21st	— — ++++
11	Kar. 53577 February 1, 1934	F. 38	4th	7th	4th 7th 12th	+ ++ ++++
12	Bri. 53162 January 10, 1934	M. 8	4th	4th	5th 13th	++++ —
13	Gra. 54472 March 25, 1934	F. 31	4th	9th	4th 10th	++ ++++
14	Cla. 53856 February 18, 1934	M. 68	4th	9th	6th 12th	++ ++++
15	Cun. 54007 February 28, 1934	F. 45	3d	5th-7th	3d 12th 18th	++ + +
16	Hor. 53792 February 14, 1934	F. 56	6th	23d	6th 16th 18th 22d 24th 35th	— — — — — —



detail (1). Briefly summarized, a test consists in mixing plasma from the patient's blood with a highly fibrinolytic living broth culture of streptococci. Coagulation of the plasma-culture mixture is induced by the addition of  $\text{CaCl}_2$ . The tubes are then placed in the water bath at  $37.5^\circ \text{C}$ . and the time between clot formation and dissolution is noted. The activity of the test culture (Strain Co) was always established by the use of plasma clot from the blood of a normal individual. The cultures, which were utilized in testing the patient's blood, always dissolved the coagulum of normal blood in less than fifteen minutes.

A measure of the resistance to lysis, which may occur with the fibrin clot of a patient's plasma, has been obtained by determining the time required for liquefaction. Since the interval varied with the blood of different patients, the degrees of resistance have been graded in the following manner.

++++	indicates	no dissolution	in 24 hours.
+++	"	dissolution	in 8 to 24 hours.
++	"	"	" 3 to 8 hours.
+	"	"	" 1 to 3 hours.
—	"	"	" less than 1 hour.

The above schema is used in the Tables of this article.

#### *Cases of erysipelas*

The data concerning these sixteen cases are recorded in Table I. All of these patients recovered.

Thirteen (81 per cent) of the sixteen patients with erysipelas either developed resistance to the streptococcal fibrinolysin during the period of observation or exhibited a high degree of insusceptibility at the time of obtaining the first sample of blood.

One patient, Number 15, possessed a moderate degree of resistance on the third day of disease, but it decreased during convalescence. This patient was treated with anti-erysipelas serum.

Two patients, Numbers 8 and 16, developed no demonstrable antifibrinolytic response during the stay in the hospital. It is interesting to note that one of these patients (Number 16) had a second attack of erysipelas immediately following the first. Ultimate recovery was, however, complete, even though the usual response was not detected. The illness in the other patient (Number 8), who failed to develop an appreciable resistance to lysis,

was not unusually prolonged. Her recovery was complete and uneventful.

From an analysis of the results in Table I with respect to the time of the appearance of anti-fibrinolytic resistance in relation to the course of the disease, it can be noted that twelve of the sixteen patients possessed the property (rated ++ or stronger) at or about the time of recovery. This relationship was approximated regardless of the duration of the illness, the two extremes of which are exemplified by Patient Number 12 who recovered on the fourth day and had an anti-fibrinolytic titer of ++++ on the fifth day, and by Patient Number 7 who recovered on the seventeenth day and had an antifibrinolytic titer of +++ on the eighteenth day.

In spite of the frequency with which anti-fibrinolytic resistance closely attends recovery, its appearance in erysipelas is occasionally delayed until convalescence is advanced. An example of this can be noted in the course of events in Patient Number 10 who began to improve on the eleventh day but his blood possessed no antilytic property until the twenty-first day.

#### *Cases of scarlet fever, acute tonsillitis, and peritonsillar abscess*

Each of the seventeen cases, included in this group, had an illness characterized by acute onset, relatively short febrile course, and uneventful recovery. The results are presented in Table II. Hemolytic streptococci of the beta type were the predominant organisms present in throat cultures from each of the patients.

Eleven (64 per cent) of the seventeen patients either developed antifibrinolytic properties during the stay in the hospital or possessed the specific resistance to a high degree at the time of obtaining the first sample of blood. Blood from the remaining six, however, did not inhibit lysis even though tests were made with specimens obtained late in convalescence.

The frequency with which patients having acute upper respiratory infections developed antilytic resistance was less (64 per cent) than that found with the erysipelatos group (81 per cent). A second difference between these two groups was noted by the fact that antilytic properties were usually not demonstrable in the blood of patients with acute infections of the throat (for example,

TABLE II  
Cases of scarlet fever, acute tonsillitis, and peritonsillar abscess

Case number	Patient. History number. Date of admission	Sex and age	Disease	Approximate day of recovery	Fibrinolytic test	
					Day of disease (bleeding)	Result
1	Rav. 52175 November 8, 1933	years F. 23	Scarlet fever	9th	2d 9th 14th 21st	— — ++ ++++
2	Den. Not hospitalized Case	M. 12	Scarlet fever	5th	5th 15th 21st	— ++ ++++
3	Owi. 52432 November 23, 1933	F. 5	Scarlet fever	5th-8th	6th 33d	++++ +
4	Jon. 51844 October 19, 1933	F. 15	Scarlet fever	7th	7th 14th 21st 27th	— — — —
5	Kea. 53804 February 15, 1934	M. 7	Scarlet fever	9th-12th	3d 14th 24th 44th	— — — —
6	Scø. 52522 November 30, 1933	M. 43	Peritonsillar abscess	10th-13th?	3d 8th 12th	— ++ ++++
7	Shi. 52293 November 15, 1933	M. 20	Peritonsillar abscess	13th	6th 15th 24th	++++ + +
8	Sha. 55051 April 23, 1934	M. 27	Peritonsillar abscess	12th	6th 15th 33d	— — —
9	Mer. 52433 November 23, 1933	F. 22	Acute tonsillitis	6th	3d 10th 20th	+ + ++++
10	Lan. 54312 March 18, 1934	F. 23	Acute tonsillitis	6th	4th 9th 24th	+ + ++++
11	Hec. 52886 December 26, 1933	M. 26	Acute tonsillitis	7th	4th 12th	++++ ++++
12	Gei. 2768 December 12, 1933	M. 30	Acute tonsillitis	3d	3d 8th 23d	— — +++
13	Hen. 54587 March 31, 1934	F. 28	Acute tonsillitis	10th	4th 10th 21st	— — ++++
14	Ber. 8458 October 23, 1933	F. 34	Acute tonsillitis	5th	3d 8th 18th	+ ++++ ++++
15	Vol. 53252 January 15, 1934	M. 32	Acute tonsillitis	4th	3d 27th	— +
16	Bla. 51994 October 30, 1933	M. 22	Acute tonsillitis	5th	3d 9th 18th 30th	— — — —
17	Gar. Not hospitalized Case	M. 28	Acute tonsillitis	5th	5th 18th 27th 36th 45th	— — — — —

Number 1 and Number 12) promptly at the time of recovery but appeared from the second to fourth week in convalescence. The delayed response, often encountered in patients with acute streptococcus infections of the throat, was noted in the former report (4) and seems to be characteristic of the upper respiratory type of infection.

*Cases of severe, progressive suppurative infections, and septicemias*

In contrast to the patients just described, in whom the illness was brief and recovery occurred early and was complete, the cases, twelve in number, which will now be presented, had severe infections. Seven of them died. Ten had septicemia.

In Table III the results of tests with the plasma clot from the patients are given together with brief notation of relevant clinical data.

From Table III it can be seen that the blood from only three (25 per cent) of the twelve patients in this group was capable of significantly retarding the fibrinolytic bacterial activity. It is also noteworthy that all three of the patients who developed antifibrinolytic properties, recovered. Two of the three had septicemia and the antilytic response was demonstrable in the period of time during which the general infection disappeared. The observations were not made, however, with sufficient frequency to determine the exact time relationship between the cessation of septicemia and the appearance of the antifibrinolytic effect.

Of the remaining nine patients, none possessed humoral, antifibrinolytic properties. Seven of them died of the streptococcus infection. Some of the fatal cases died very soon after the beginning of the infection and so may have succumbed before the response could be elicited.

The two patients (Numbers 1 and 2), who recovered, but in whose blood antifibrinolytic properties were not detectable, were of special interest. They were followed through long severe illnesses and also during convalescence. In no test with the blood of either patient did the rate of clot dissolution differ from that of normal blood. It is of interest to note, however, that the reactivation of the infection occurred in the third to fourth week after the beginning of the first signs of the illness. In the course of the self limiting

acute streptococcus infections of the upper respiratory tract (Table II) it has just been noted that this late period was found to be the time at which antifibrinolytic resistance frequently appeared. The possibility is suggested, therefore, that the liability to exacerbation or continuation of infection is greater among patients without antilytic resistance than in those who develop the capacity to inhibit fibrinolytic activity.

When the data presented in this article are analyzed with respect to the activity of the infection in relation to the presence or absence of antifibrinolytic properties in the blood, the following results are obtained:

Of thirteen patients, who did not possess antilytic properties *within three weeks* after the first manifestations of infection (Patients Numbers 8 and 16 in Table I, Numbers 4, 5, 8, 15, 16, and 17 in Table II, and Numbers 1, 2, 3, 6, and 9 in Table III), two died (Numbers 6 and 9 in Table III) and four had recrudescences and prolongation of the illness (Number 16 in Table I, and Numbers 1, 2, and 3 in Table III). In contrast to this result, none of the twenty-eight patients, who developed resistance to streptococcal lysis, showed any evidence of continued bacterial invasion after the specific response was established.

*Cases of acute rheumatic fever and acute hemorrhagic nephritis*

Of the thirteen patients, who comprise this group, each had signs and laboratory findings indicating active rheumatic state or renal involvement. In addition, each case either gave the usual history of some local infection preceding the visceral affection or presented, at the time of admission, evidence of such an infection. The site of the activating infection is given in Table IV. From four of the eight rheumatic patients and from each of the nephritic cases, hemolytic streptococci of the beta type were recovered. Since repeated throat cultures were not made on the four rheumatic patients who were negative for hemolytic streptococci on one or two occasions, the bacteriological findings may be inconclusive.

The clinical course of the patients outlined in Table IV, is given with respect to day of disease from the standpoint of the local acute infection, and not on the basis of the rheumatic fever or

TABLE III  
Cases of septicemia and severe suppurative infections

Case number	Patient. History number. Date of admission	Sex and age	Initial infection	Complications	Final outcome	Day of disease	Blood culture*	Fibrinolytic test
1	Sta. 51946 October 27, 1933	years F. 5	Scarlet fever 4 weeks ago	Otitis media, mastoiditis, sinus thrombosis, multiple abscesses	Recovered. Temperature normal about 85th day	25th 45th 55th 70th 80th 90th 115th	— 80 + 380 + —	— — — — — — —
2	Hos. 54874 April 14, 1934	M. 12	Otitis media 18 days ago	Mastoiditis, sinus thrombosis, osteomyelitis	Recovered. Temperature normal about 45th day	20th 25th 30th 45th 65th	— 3 — — —	— — — — —
3	Wal. 81278 December 8, 1933	F. 10 mos.	Upper respiratory infection 7 days ago	Adenitis, osteomyelitis, multiple abscesses	Recovered. Temperature normal about 50th day	15th 25th 45th 65th 120th	500+ 70 —	— — — ++++ —
4	Woo. 51424 March 23, 1934	F. 58	Otitis media 6 days ago	Erysipelas, mastoiditis	Recovered. Temperature normal about 17th day	10th 15th 20th 30th	+ —	— + ++++ ++++
5	For. 53143 January 9, 1934	F. 25	Acute tonsillitis 2 weeks ago	Pneumonia (pneumococcus Group IV), empyema (hemolytic streptococci)	Recovered. Acute process subsided about 20th day	15th 25th 40th 120th	—	+ ++++ ++++ ++++
6	Bea. 54716 April 6, 1934	M. 8	Otitis media 10 weeks ago	Mastoiditis, brain abscess	Died 90th day	75th 85th	—	— +
7	Coy. 29871 December 18, 1933	F. 41	Cervical adenitis 5 days ago	Abscess of neck	Died 9th day	6th 8th	2 15	— —
8	Wil. 54087 March 1, 1934	F. 36	Abortion 2 days ago	Puerperal sepsis	Died 15th day	5th 14th	+ +	— —
9	Fle. 21184 March 30, 1934	F. 23	Postpartum 6 days	Puerperal sepsis	Died 27th day	9th 18th 25th	40 + +	— — —
10	Mol. 52816 December 20, 1933	F. 20	Acute tonsillitis 4 days ago	Peritonitis	Died 8th day	5th	2	—
11	Nea. 53549 February 9, 1934	F. 21	Postoperative	Wound infection	Died 7th day	4th	+	—
12	Bai. 53830 March 5, 1934	F. 63	Postoperative	Wound infection	Died 7th day	3d 6th	105 1000	— —

\* Numerals indicate number of colonies of hemolytic streptococci per cc. of blood.

+ sign indicates positive culture not measured quantitatively.

— sign indicates negative culture.

TABLE IV  
Cases of active rheumatic fever and acute hemorrhagic nephritis

Case number	Patient. History number. Date of admission	Sex and age	Rheumatic fever	Local infection			Fibrinolytic test		Rheumatic state at time of last test
				Site	Duration of symptoms	Culture*	Day of disease (local infection)	Result	
1	Lew. 30093 November 23, 1933	years F. 17	Recurrent for 12 years	Throat	5 days	Positive	6th 18th 30th	— ++ ++++	Improved, not quiescent
2	Gil. 52627 December 6, 1933	M. 16	First acute symptoms 3 weeks ago	Throat	3 weeks	2 cultures negative	21st 30th 45th 55th	— ++ + —	Quiescent
3	Cor. 54357 March 19, 1934	M. 39	First acute symptoms 1 month ago	Throat	3 weeks	1 culture negative	27th 55th	+++ +++	Improved, not quiescent
4	Dec. 23642 November 7, 1933	M. 32	Recurrent for 7 years	Upper respiratory tract	1 month (mild)	Positive	21st 35th	++++ +++	Improved, not quiescent
5	Col. 35136 May 14, 1934	F. 16	Recurrent for 3 years	Throat	1 month	Positive	35th 55th	+++ ++	Quiescent
6	Gro. 52448	F. 13	Chorea 5 years ago. Polyarthritis 3 weeks ago	?	?	1 culture negative	30th? 55th?	++ +	Quiescent
7	Sch. 55180 April 30, 1934	F. 14	Recurrent for 6 years	Throat	6 weeks	1 culture negative	40th 50th 120th	++++ ++++ +++	Unimproved
8	Rol. 55651 May 22, 1934	F. 14	First symptoms 2 weeks ago	Throat	3 weeks	Positive	30th 55th 85th	++++ +++ —	Quiescent
Acute nephritis									Acute nephritis
1	Yan. 46383 January 1, 1934	F. 19	First signs 14 months ago 2nd admission	Throat	3 days	Positive	4th 18th 30th 45th	— ++++ ++ +	Improved
2	Ger. 52952 December 27, 1933	F. 15	Edema appeared 5 days ago	Finger	2 weeks	Positive	15th 35th 55th	++++ ++++ ++++	Improved
3	McK. 53939 March 18, 1934	M. 33	Edema appeared 5 months ago	Furunculosis (recurrent)	9 months	Positive	On Adm. 21st? 35th?	++ ++ +	Slightly improved
4	Pai. 52292 November 15, 1933	F. 45	Hematuria on admission	Erysipelas	6 days	Positive	6th 18th 30th 55th	— ++ ++ —	Improved
5	Sho. 54066 March 4, 1934	M. 24	Appeared after admission	Peritonissillar abscess	5 days	Positive	6th 15th 27th 55th	— — — —	Improved

\* Refers to presence or absence of hemolytic streptococcus of the beta type.

nephritis. In a few instances, where the data are uncertain, the questionable factors are indicated.

*Rheumatic fever.* The results obtained with the eight cases of rheumatic fever (Table IV), demonstrate that resistance was present in the first specimen of blood or developed during the period of observation. The appearance of antifibrinolytic properties in patients with rheumatic fever seemed to follow the acute upper respiratory infection with about the same regularity as has been found in uncomplicated cases of acute tonsillitis, and was equally as definite in the patients from whom hemolytic streptococci were not recovered as in the others having positive cultures. Hadfield, Magee and Perry (5) have reported the results of observations on the antifibrinolytic resistance in a large number of patients with active or latent rheumatic fever. These investigators found that the plasma clot from cases of active disease was frequently resistant to lysis, whereas the fibrin from the blood of quiescent cases was susceptible. They further indicate that the determination of the presence or absence of the antifibrinolytic effect of the blood may be helpful in estimating the activity of the rheumatic state. The results obtained in this laboratory with a small group of patients confirm the findings of Hadfield and his associates.

*Acute hemorrhagic nephritis.* Each of the five cases of acute nephritis (Table IV) had a local infection from which hemolytic streptococci of the beta type were isolated. The plasma clot from four of the patients was definitely resistant. The fibrin from the blood of one patient (Sho.), who had peritonsillar abscess followed later by hematuria, was found to be susceptible in each of five tests performed at intervals for about seven weeks.

The presence of antifibrinolytic properties in the blood of patients with acute nephritis appears to follow the activating streptococcus infection in a manner comparable to the specific response evoked by acute diseases not complicated by renal involvement.

In summarizing all of the observations which are included in this article, the following results emerge:

Of thirty-eight patients who recovered from acute streptococcus infections, twenty-eight (73.6 per cent) either possessed at the time of admis-

sion, or subsequently developed, a definite degree of antifibrinolytic resistance.

Of seven cases dying from infection by hemolytic streptococci, the fibrin clot from each was susceptible up to the time of death.

Of eight patients with acute rheumatic fever, the plasma clot from each was resistant during active infection.

Of five patients with acute hemorrhagic nephritis, four possessed antifibrinolytic resistance.

#### DISCUSSION

The frequency with which recovery from acute hemolytic streptococcus diseases is followed by the appearance in the circulating blood of a specific antistubstance directed against the fibrin dissolving action of streptococci, demonstrates the fact that the body takes cognizance of the fibrinolytic property of the infecting organism.

The results, which are presented in this report, concern the occurrence of the antifibrinolytic response in the blood of patients in relation to the course of the illnesses. For this purpose, cases were selected for study which presented different types of acute streptococcus infections of different degrees of severity. The diseases ranged from mild self limiting processes to more serious types of general infection or metastatic suppuration. The results demonstrate that most of the patients (73.6 per cent) who recovered possessed humoral antifibrinolytic properties. On the other hand, a few others overcame the infection without evoking an antilytic response. The development of antifibrinolysin, therefore, as measured in the circulating blood, although a common occurrence, was not found to be a universal sequel to acute streptococcus diseases. It is interesting to note, however, certain striking differences between the course of the diseases in the patients, who developed antifibrinolytic activity, and others, whose blood was devoid of this property. For example, antifibrinolysin was found in the blood of those individuals only whose recovery was complete and whose convalescence was not interrupted by additional reactivation of the streptococcus infection. On the other hand, antifibrinolysin was not detected in the blood of cases of fatal infection, and did not appear at the usual time (first to fourth week) in others with metastasizing suppurative processes.

auscultation during immersion was continued for five minutes. At the end of this time observations on the capillary pulsation were again made and systolic and diastolic blood pressures again measured.

from a sharp loud to a low muffled sound. Care was taken to maintain the temperature of the water at 114° F. by adding hot water when necessary.

The capillary pulsation immediately before and

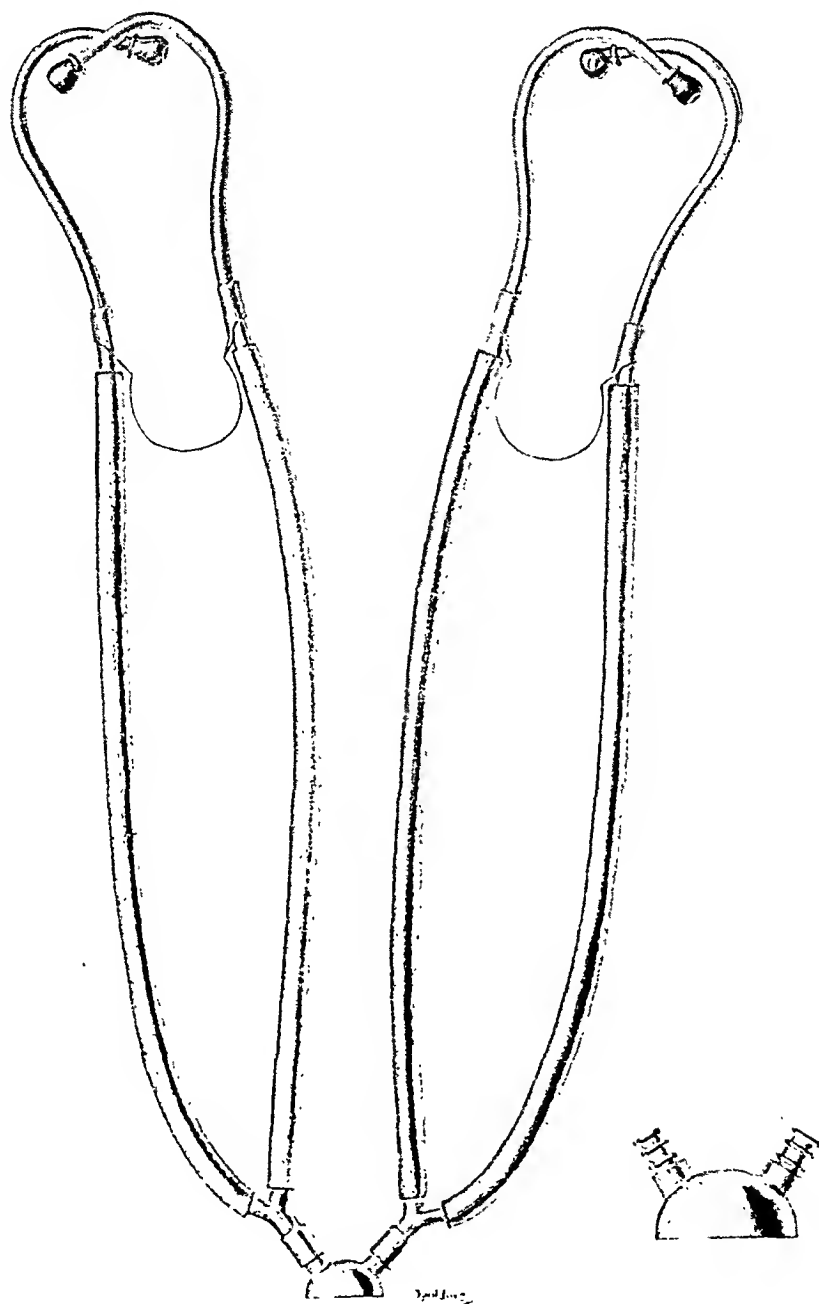


FIG. 1. ARRANGEMENT OF STETHOSCOPE

PEABODY BELL.

The standard mercury sphygmomanometer with cuff 14 cm. wide was used. All measurements were made within the minute immediately preceding and following the immersion of the forearm in hot water. The arterial diastolic pressure reading was taken at the point of change

after immersion was sought by pressing with a glass slide over the pads of the distal phalanges of the fingers. Each observation was checked by two observers, one of whom counted the alternate waxing and waning of the pulsation, while the other checked this with the patient's pulse. In

each case the observer's own pulse was eliminated as a possible determining factor.

Only diastolic murmurs that could be maintained indefinitely and that filled or practically filled the entire duration of diastole were considered typical of Duroziez's sign. In the older age groups a definite diastolic murmur produced in the usual manner and heard during many successive cycles generally lasted only through approximately three-fourths of diastole. Such murmurs were included as positive. Throughout the study the patient was at rest. Enough observations were made on different days to be certain of the constancy of findings for each patient.

### OBSERVATIONS

#### A. Duroziez's sign in normal subjects<sup>3</sup> and in patients with arterial hypertension

*Variation with age.* In subjects with no evidence of cardiovascular disease the incidence of the artificially produced Duroziez's sign is greatest in young subjects and decreases with advancing years (Figure 2). In subjects between the ages of 20 and 40 years, Duroziez's sign was practically always obtainable; in middle age, 40 to 60 years, the incidence dropped sharply to about 40 per cent; past 60 years of age, the incidence gradually declined further, and in patients beyond the age of 70, Duroziez's sign of the peripheral type was never obtainable.

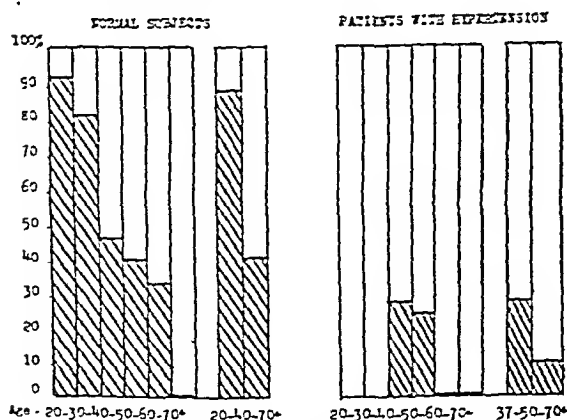


FIG. 2. DUROZIEZ'S SIGN IN NORMAL SUBJECTS AND PATIENTS WITH HYPERTENSION.

Variation in percentage incidence of Duroziez's sign with age.

<sup>3</sup> For the purpose of this study patients with no evidence of cardiovascular disease are considered normal.

In patients with arterial hypertension the incidence of Duroziez's sign of the peripheral type was generally lower than in subjects with normal arterial pressure; and the incidence declined more sharply toward zero as age advanced (Figure 2). The lowered incidence in arterial hypertension is even more striking when the 42 normal subjects are contrasted with the 41 patients with hypertension of similar ages (Table I).

TABLE I

*Incidence of Duroziez's sign according to age*

Age  years	Normal subjects		Patients with arterial hypertension		
	Number of subjects	Positive Duroziez	Number of subjects	Positive Duroziez	
		Subjects   Per cent		Subjects   Per cent	
40 to 60	33	14   42	24	6   25	
60 to 73	9	3   33	17	0   0	
Total and averages	42	17   40	41	6   14	

*Duroziez's sign and pulse pressure.* No relation between the magnitude of pulse pressure and the incidence of Duroziez's sign was found in normal persons under the age of fifty. Above that

TABLE II

*Incidence of Duroziez's sign according to pulse pressure*

Pulse pressure  mm. Hg	Normal subjects						Patients with arterial hypertension		
	Under 50 years			Over 50 years			All ages		
	Duroziez's sign			Duroziez's sign			Duroziez's sign		
	+	-	Percentage positive	+	-	Percentage positive	+	-	Percentage positive
	+	-	Percentage positive	+	-	Percentage positive	+	-	Percentage positive
20 to 40	11	6	64	8	4	66	—	—	—
40 to 60	18	8	69	0	10	0	4	4	50
60 to 80	—	—	—	—	—	—	1	10	9
80 to 100	—	—	—	—	—	—	1	10	9
100+	—	—	—	—	—	—	0	10	0

age, however, and in hypertensive subjects of all age groups, it was found that the greater the pulse pressure, the lower the incidence of Duroziez's sign (Table II).

*Duroziez's sign and diastolic blood pressure.*



The incidence of Duroziez's sign in normal subjects with diastolic blood pressures below 80 mm. of mercury was compared with that in normal subjects with pressures above 80 mm. of mercury. Taking into account the influence of age, those with the higher diastolic pressure showed Duroziez's sign more frequently. This relationship was even more pronounced in the patients with arterial hypertension (Table III).

TABLE III

*Incidence of Duroziez's sign according to diastolic blood pressure*

Diastolic blood pressure	Normal subjects			Patients with arterial hypertension		
	Duroziez's sign			Duroziez's sign		
	+	-	Percentage positive	+	-	Percentage positive
mm. Hg						
60-80	15	15	50	-	3	0
80-100	20	10	66	0	12	0
100-120	—	—	—	3	13	19
120+	—	—	—	3	6	33

*Duroziez's sign and pulse rate.* We found no relation between the radial pulse rate and the incidence of Duroziez's sign. Immersion of the arm had no apparent effect on the pulse rate or on the systolic or diastolic arterial pressure.

#### *B. Capillary pulsation in normal subjects and in patients with arterial hypertension*

*Capillary pulsation after immersion.* Capillary pulsation was present after immersion in all cases of all groups. This finding differs somewhat from the observation of Lewis (2). He states that there is an obvious, though not wholly constant relation between age and exhibition of capillary pulsation in the heated skin. "Subjects ranging in age from seventeen to thirty-five display capillary pulsation most fully and with much uniformity in its degree. As age advances into and beyond the forties it becomes less evident than in young people and many are found in which it is but slight . . . or in which it is altogether absent." We have never found it absent in the heated skin and have found only a slight, if any, relationship between intensity and age.

*Incidence of spontaneous capillary pulsation.* It is of interest to note the number of subjects showing spontaneous capillary pulsation. Of sixty-three normal subjects, nineteen, or 30 per cent, were positive; of forty-one patients with hypertension twenty-two, or 55 per cent, were positive. Almost all of these patients had normal body temperature and were examined under similar external conditions, the room temperature varying only from 74° to 76° F.

*Spontaneous capillary pulsation and age.* In the normal subjects no relationship between age and the presence of spontaneous capillary pulsation was apparent. In the hypertensive group, however, the older age groups showed a greater incidence of spontaneous capillary pulsation (Figure 3). This was attributable not to age, per se,

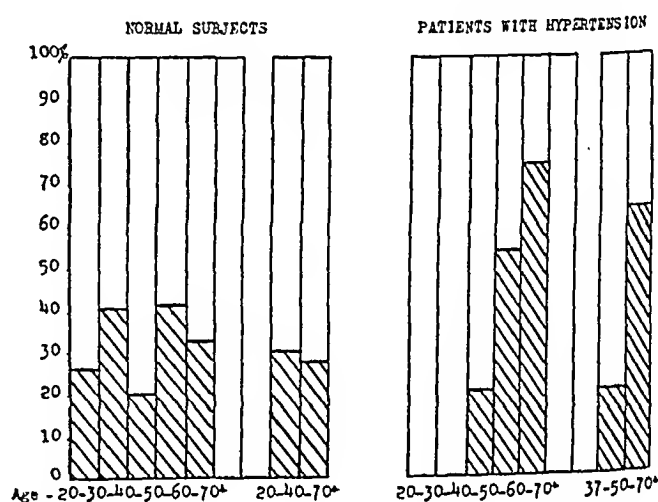


FIG. 3. CAPILLARY PULSATION IN NORMAL SUBJECTS AND PATIENTS WITH HYPERTENSION.

Variation in percentage incidence of spontaneous capillary pulsation with age.

but rather to the fact that the older subjects in the group showed the widest pulse pressures.

*Spontaneous capillary pulsation and pulse pressure.* Both in the normal and hypertensive subjects the incidence of spontaneous capillary pulsation varied directly with the magnitude of pulse pressure, as seen below.

Pulse pressure	Normal subjects with spontaneous capillary pulsation	Subjects with arterial hypertension with spontaneous capillary pulsation
mm.	per cent	per cent
20-60	27	30
60-80	75	56
80+		75
Average	30	55

The relatively greater incidence of spontaneous capillary pulsation in hypertension is due, we believe, to the wider pulse pressures so frequently seen in this disease.

*Spontaneous capillary pulsation and diastolic blood pressure.* The height of the diastolic blood pressure per se did not seem to influence the incidence of spontaneous capillary pulsation. In both hypertensive and normal groups spontaneous capillary pulsation was often found in patients with low diastolic pressure. This was probably due to the larger pulse pressures in such subjects.

#### MISCELLANEOUS GROUP

In view of the well known fact that the velocity and volume of blood flow frequently are increased in anemia and that this would theoretically predispose to the presence of Duroziez's sign, four cases with primary anemia and two cases with secondary anemia were studied. The three youngest patients showed Duroziez's sign after immersion of the arm and the other three did not.

Two cases of polycythemia vera with hypertension showed no Duroziez's sign, which was in accord with our findings in patients with hypertension. In both, spontaneous capillary pulsation was present.

One case each of thrombo-angiitis obliterans and Raynaud's disease, interestingly enough, showed a positive Duroziez's sign. In both, signs and symptoms of the disease were referable only to the lower extremities.

#### COMMENT

Until recently, the sign originally described by Duroziez, namely, a diastolic murmur heard over the larger peripheral arteries,<sup>4</sup> was always associated with aortic insufficiency and was attributed to a diastolic reflux of blood found in that condition. Of late it has been shown that this sign is not pathognomic of that disease, inasmuch as it has been demonstrated to be present in other conditions, and, in fact, can be artificially produced in normal individuals. Under such artificial con-

ditions where aortic reflux is not a factor, Duroziez's sign has been shown to be due to an increase in peripheral blood flow dependent upon the extent of peripheral vasodilatation. To distinguish this type of Duroziez's sign from that present in aortic regurgitation, we have termed it "the peripheral type." When, therefore, Duroziez's sign of the peripheral type is not present spontaneously and cannot be artificially induced, it may be assumed that the peripheral blood flow in the part studied cannot be sufficiently increased. Other factors being constant, either or both of the following situations may be present: (1) the minute vessels cannot dilate sufficiently to permit the increased forward flow of blood necessary to produce Duroziez's sign. (2) The number of minute vessels is decreased, even though those remaining are capable of dilating. The extent of the peripheral vascular bed would thus be decreased. Under such conditions changes in peripheral blood flow might still occur but the blood flow would be insufficient to produce Duroziez's sign. However, Weiss and Frazier (4) have shown that the number of minute vessels of the skin is probably not diminished. The first mechanism would, therefore, seem to be the more important.

The decreasing incidence of Duroziez's sign of the peripheral type with advancing years signifies a gradual reduction in the peripheral vascular bed due either to spasm or sclerosis of the minute vessels. Since local heat used in the elicitation of this sign abolishes spasm, the lowered incidence of Duroziez's sign is presumably due to a relative inability of the minute vessels to dilate or to a decrease in their number. The generally lowered incidence of Duroziez's sign in patients with arterial hypertension is in harmony with the clinical and pathological observations of predominant involvement of the arterioles in that condition.

Our studies were confined to the forearm and, although arteriolar sclerosis in muscle and skin according to certain pathological studies is considered rare, the present clinical studies of Duroziez's sign suggest that the peripheral functional vascular bed is affected to greater degree in such subjects than in similar persons with normal blood pressure.

Three normal subjects failed to show a positive Duroziez's sign, and six patients with arterial

<sup>4</sup> Duroziez's original description referred to a diastolic murmur heard over the femoral artery, but clinical usage has sanctioned the wider application of this sign to a diastolic murmur heard over any of the larger peripheral vessels.



# AN OPTIMAL DIET IN PROMOTING NITROGEN GAIN IN NEPHROSIS<sup>1</sup>

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(Received for publication July 14, 1933)<sup>2</sup>

The present state of our knowledge of nephrosis leaves very little doubt that the level of plasma proteins is one of the important factors that control the fluid distribution between blood and tissues. According to the work of Moore and Van Slyke (1) the relations between plasma protein content and nephrotic edema are such that the critical point below which edema occurs is approximately 2.5 per cent albumin and 5.5 per cent total proteins. This has been found generally true both in pure nephrosis and in the nephrotic stage of chronic hemorrhagic nephritis. Similarly through a deficit in plasma proteins, edema may be brought about in dogs by plasmapheresis (2, 3) and occurs in man with malnutrition (4, 5, 6, 7). While the plasma protein deficit in plasmapheresis and nutritional edema can be easily explained, the cause of low plasma protein in nephrosis is more obscure. Epstein (8, 9), Kollert (10) and others attribute the low albumin content to direct loss of albumin in urine, while Peters, Bulger et al. (11) emphasize the importance of protein deprivation or starvation as an additional factor in the production of plasma protein deficit in nephrosis. Through lack of appetite or through misdirected management patients with this disease receive a diet containing proteins far short of their needs.

With a realization of the importance of a plasma protein deficit and its contributing causes, attempts have been made to raise plasma proteins by dietary means. Epstein (8) was the first to advocate a high protein diet in the treatment of nephrosis and clinical observation convinced him of the efficacy of the treatment. The use of a high protein diet was supported by the work of

MacLean (12) and Worley (13). Others, however, are less favorably impressed by Epstein's diets. Linder, Lundsgaard and Van Slyke (14) are of the opinion that diets in nephrosis should contain an adequate but not an excessive amount of protein. Peters and his colleagues (11) have defined the dietary requirements for nephritis more accurately. They conclude that besides enough protein to cover the nitrogen catabolism and that lost as albumin in urine, the patients should be given an additional amount of protein to repair the previous nitrogen wastage and, if possible, the plasma protein deficit. By the administration of large amounts of carbohydrate and fat it has been possible to reduce the protein catabolism to 0.5 to 0.7 gram per kgm. of body weight, per day.

The work reported in this paper was undertaken to discover a diet so constituted in caloric and protein intake that it would insure a maximum gain in nitrogen without necessarily imposing on the appetite of the patients. The possible effects of varying proportions of animal and vegetable proteins were also taken into consideration. Such a study requires rigid dietary control for a long period of time and may not be applicable to many nephrotic patients in the active stage when they have precarious appetites. Consequently the number of patients suitable for such a study is limited. We have two cases in which sufficient data have been obtained to permit us to draw certain conclusions in regard to the composition of what may be called an optimal diet for nephrosis.

## EXPERIMENTAL SUBJECTS

*Case 1.* J. S. Y., a Chinese boy of 10 was admitted July 1, 1928, for edema of the face, extremities and genitalia which had begun 3 weeks prior to admission. There was no history of febrile disease preceding the appearance of edema, nor was the present illness accompanied by any constitutional symptoms. He presented general anasarca, a temperature of 39.6° C., pulse 140, respiration 34, blood pressure 100/66. No foci of infection were evident in nose, pharynx, teeth, or ears. The ocular fundi were normal. There was no dullness over the lungs, but

<sup>1</sup> A preliminary report of this work appeared in *Proc. Soc. Exper. Biol. and Med.*, 1933, 30, 986.

<sup>2</sup> This paper as revised by the authors was lost in transit to the Journal about September 1, 1933. Following inquiry from the authors on January 21, 1935, the loss was discovered. A copy of the manuscript was sent to the Journal and was received January 25, 1935.

râles were heard everywhere. The heart was not enlarged and no murmurs were heard. The abdomen was distended and there was free fluid in the peritoneal cavity. The urine contained ++ albumin, no sugar; in the sediment were found many white blood cells, red blood cells and hyaline and granular casts; the guaiac test was positive. The phenolsulphonphthalein excretion was 55 per cent in two hours. There was no anemia. The leukocyte count varied between 7000 and 12,000. Blood chemical findings were as follows: nonprotein nitrogen 35 mgm., uric acid 4.7 mgm., creatinine 1.5 mgm., and cholesterol 533 mgm., per 100 cc.; plasma albumin 3.3 per cent and total proteins 5.2 per cent; carbon dioxide capacity, 54 volumes per cent. The basal metabolic rate was -9.4 per cent.

There was on admission acute bronchitis which subsided promptly with return to normal of temperature and pulse rate. Edema decreased rapidly following catharsis with magnesium sulphate. After this, edema recurred at intervals. In December 1928, at the height of edema, a right maxillary sinusitis was discovered. Puncture secured thick pus, culture of which showed *Streptococcus hemolyticus*, *Staphylococcus aureus* and *B. coli*. After repeated irrigations the sinusitis cleared up and edema disappeared. He gained 3 kgm. of body weight, without edema, in the next three months. The plasma proteins rose slightly, the albumin to 4.0 per cent and the total proteins to 5.9 per cent. Cholesterol decreased to 101 mgm. per cent. The urine, however, still contained albumin and a few red blood cells. The patient was discharged in good condition in April of 1929.

For the remainder of the year 1929, he stayed at home and did fairly well. But in the following year, 1930, he had to be readmitted to the hospital five times because of recurrences of edema. Each recurrence was apparently associated with some acute respiratory infection, such as tonsillitis, bronchitis and pharyngitis. After each admission edema subsided promptly, although slight pitting over the ankles persisted. Tonsillectomy was done in May, during the fourth admission, with practically no reaction. During the year plasma albumin varied between 1 and 2 per cent and total proteins between 3 and 5 per cent. Usually marked edema was accompanied by lower plasma proteins. Blood cholesterol varied between 300 and 500 mgm. per cent.

On the sixth admission the patient stayed in the hospital for approximately six months (from December 27, 1930, to June 15, 1931). During this period, after the initial rapid loss of edema, his condition remained almost stationary. Slight pitting edema was always present over the ankles. Plasma albumin fluctuated around 2 per cent, total proteins around 4.5 per cent, and blood cholesterol around 300 mgm. per cent. Albuminuria was constantly present to the extent of 1 to 4 grams per day. Sediment count according to the technique of Addis showed 280,000 casts, 1,700,000 red blood cells, and 11,000,000 leukocytes and epithelial cells per 12 hours. During the months of January to March, he served as an experimental subject for the study of the effects on water

balance of acids and alkalis. During March and April the effect of thyroid medication was studied. These studies will be reported separately.

In September 1931 he returned for his seventh admission at our request. He had done well in the summer and on admission there was only slight edema over both tibiae. Plasma proteins were approximately at the same level as on preceding admission, although blood cholesterol was only 150 mgm. per cent and stayed at this level. The urine showed a faint trace of albumin. A sediment count revealed 56,000 casts, 4,500 erythrocytes, and 600,000 white blood cells in 12 hours. Soon after admission he developed a febrile illness which lasted approximately three weeks. This proved to be paratyphoid A fever. He did not suffer much subjectively, although he lost approximately 2 kgm. in body weight and the plasma albumin came down to 1.5 per cent and total proteins to 3.7 per cent at the height of the illness. Of great interest was the complete disappearance of albuminuria after he recovered from the infection. A sediment count revealed approximately 2000 casts, 200 red blood cells, and 13,000 leukocytes, figures well within normal limits. He was then entirely free from edema and steadily put on weight. The plasma albumin rose rapidly to approximately 4 per cent and total proteins to 6 per cent, at which levels they remained throughout the remaining period of observation. He was, in short, considered a normal individual. His otherwise uneventful course in the hospital was interrupted by a mild attack of chickenpox in April and of pityriasis rosea in May 1932. He stayed in the hospital for approximately 9 months. From October 16, 1931, to April 24, 1932, he was studied from the standpoint of nitrogen balance on diets containing different amounts of calories and protein (see Table I) and different percentages of animal and vegetable proteins. From April 25 to June 11, he was again observed for the effect of thyroid medication so as to compare the results with those of the earlier period in which he was still in the nephrotic state.

*Case 2.* F. H. T., a farm laborer of 35, was admitted June 8, 1931, for ulceration of the skin of 10 months' duration and general anasarca of 4 weeks' duration. He was discharged improved June 13, 1932. A year prior to admission the patient had a venereal exposure followed by urethral discharge, a penile sore, and a generalized reddish papular skin eruption. These cleared up promptly. Approximately 8 months prior to admission, he noticed that many of the faded papules over the extremities, buttocks, face and scalp began to spread and become ulcerated. Various local applications were used without result. Four weeks prior to admission, edema of the lower extremities was noted and very soon his external genitalia, face, and upper extremities became involved. Scanty urination and loss of strength were accompanying symptoms. There was no fever and the appetite remained good.

Physical examination revealed a moderately sick and slightly emaciated man with temperature 37.2° C., pulse 104, respirations 20, blood pressure 90/70. Over the

right thigh, left popliteal and elbow regions, buttocks, face and scalp, there were large areas of superficial annular ulcerative lesions with atrophic scars in the center. The lower extremities, external genitalia and lower lumbar region were markedly edematous, while the forearms, backs of hands and abdominal wall were slightly so. He had advanced trachoma with ectropion of the left lower eyelid. The pupillary reactions and ocular fundi were normal. The epitrochlear and inguinal lymph nodes were palpable. The lungs were clear, although the bases were high. The heart showed no abnormality. The abdomen was distended, with bulging of the flanks, a fluid wave and shifting dullness.

*Laboratory examinations.* The urine contained +++ albumin with many hyaline and granular casts and leukocytes. No red blood cells were noticed on repeated examination. A smear of the sediment showed gram-negative diplococci, both extra- and intracellular. The phenolsulphonphthalein excretion was 15 per cent in 2 hours, the urea clearance test, 35 per cent of normal. A sediment count (Addis) showed white blood and epithelial cells to be 1.4, red blood cells 0.207 and casts 0.726 million per 12 hours. Blood counts revealed slight anemia with a normal number of white blood cells. Blood Wassermann and Kahn tests were both strongly positive. The blood nonprotein nitrogen was 65 mgm. per cent, cholesterol 208 mgm. per cent, plasma CO<sub>2</sub> capacity 45.8 volumes per cent, plasma albumin 1.11 per cent and total proteins 3.15 per cent. Stools contained trichomonas and ova of ascaris and hookworm. The basal metabolic rate was -4.3 per cent.

The main diagnoses in this case were nephrosis and syphilis with skin manifestations. Chronic gonococcal urethritis, ankylostomiasis, ascariasis and trichomoniasis were incidental conditions.

Anti-syphilitic treatment was commenced shortly after admission and the skin lesions healed completely after the third injection of neoarsphenamine. The injections were continued at weekly intervals until he had received 22 injections of neoarsphenamine and 14 of sodium potassium bismuth tartrate. His blood Wassermann reaction became negative on October 7, 1932, 16 months after the commencement of treatment.

No special treatment was instituted for his renal condition except a diet that contained 120 grams of proteins and 2200 to 3200 calories with limited amounts of fluid and salt. His edema remained stationary until about 3 weeks after admission when it began to subside. Within the next 6 to 7 weeks, his body weight decreased from 57.6 to 43.4 kgm., a decrease of 14 kgm., resulting in the disappearance of gross edema. Slight pitting edema over the tibiae could still be demonstrated for the next 5 months, after which he became entirely free from edema. He steadily put on flesh so that a maximum weight of 60 kgm. was reached 9 months after gross edema had disappeared. During the period of rapid discharge of edema his plasma albumin was 1.5 per cent and total proteins 3.1 per cent. These gradually increased so that his plasma albumin became 3.5 per cent and total proteins 6.0 per cent 7 months after admission, when edema had

entirely disappeared. After that the plasma proteins stayed at these levels throughout the period of observation. Albuminuria also improved, though slowly. It averaged 12 grams per day on admission and decreased to 0.5 gram per day on discharge. Occasional hyaline casts were still present on discharge. The phenolsulphonphthalein excretion was 75 per cent on discharge, the blood pressure 110/60.

From September 30, 1931, to April 24, 1932, the patient was on a rigidly controlled regime for the study of nitrogen balance in relation to the intake of protein and calories. From April 25 to June 11, 1932, thyroid was administered to ascertain its effect on the nitrogen and respiratory metabolism during the symptom-free period of nephrosis.

A follow-up examination on October 7, 1932, showed the patient to be in good nutrition without any evidence of edema. The urine contained a trace of albumin, occasional hyaline casts and a few leukocytes. The urea clearance test was 92 per cent of normal, the blood non-protein nitrogen 47 mgm. per cent, plasma albumin 3.72 per cent and total proteins 5.91 per cent.

#### PROCEDURE

The patients had been placed in the Metabolism Ward for some time before metabolism studies were commenced. Diets were accurately prepared and served, and stools and urine completely collected.

*Diets.* The food values were taken from the compilations of Wu (15), based on analysis of local articles of food. Tables I and II give the composition of various diets taken by the patients. Each diet was used for two to three periods of 4 days each, but occasionally observations on a given diet were extended to four or five periods. As indicated in the tables, the general plan was to study the effect of three variables in the diets, namely, caloric intake, protein intake, and variations in the proportion of animal and vegetable proteins. When the effect of one variable was studied, the other two were held constant. From Table I it can be seen that Periods 5 to 21 were devoted to the study of the effect of variations in caloric intake from 1500 to 2590, with an approximately constant protein intake of 50 grams, of which 50 per cent were of animal origin. Periods 22 to 32 were used to study the effect of varying the protein intake from 50 to 125 grams, with a constant caloric intake of 2310 and 50 per cent of animal protein. Periods 1 to 4 and 33 to 47 are to be considered together. During these the effect of varying the percentage of animal and

TABLE I  
Case 1. Composition of diets

Period number	Date	Protein	Animal protein	Fat	Carbohydrate	Calories	Salt	Average body weight	Protein per kgm.	Calories per kgm.	Calories from protein
		grams	per cent	grams	grams		grams	kgm.	grams		per cent
1-2	Oct. 16-23	24.6	0	50	298	1741	6	25.8	0.95	67	5.6
3-4	Oct. 24-Nov. 4	25.2	92	50	300	1750	6	26.0	0.97	67	5.8
5-7	Nov. 5-16	50.2	50	50	275	1751	6	27.1	1.85	65	11.5
8-9	Nov. 17-24	50.1	50	70	300	2030	6	28.0	1.79	73	9.9
10-11	Nov. 25-Dec. 2	50.3	50	90	325	2311	6	28.5	1.76	81	8.7
12-13	Dec. 3-10	50.2	50	110	350	2591	6	29.4	1.71	88	7.7
14-15	Dec. 11-18	50.0	50	90	325	2310	6	29.7	1.68	78	8.7
16-17	Dec. 19-26	50.0	50	70	300	2030	6	29.7	1.68	68	9.9
18-19	Dec. 27-Jan. 3	50.0	50	50	275	1750	6	29.7	1.68	59	11.5
20-21	Jan. 4-11	50.0	50	40	235	1500	6	29.4	1.70	51	13.3
22-23	Jan. 12-19	50.1	50	90	325	2310	6	29.6	1.69	78	8.7
24-26	Jan. 20-31	74.9	50	90	300	2310	6	30.1	2.49	77	13.0
27-29	Feb. 1-12	100.0	50	90	275	2310	6	30.5	3.28	76	17.3
30-32	Feb. 13-24	125.3	50	90	250	2311	6	30.9	4.05	75	21.6
33-35	Feb. 25-Mar. 7	74.8	50	90	300	2309	6	31.1	2.41	74	13.0
36-38	Mar. 8-19	75.2	25	90	300	2311	6	31.5	2.41	73	13.0
39-42	Mar. 20-Apr. 4	74.8	0	90	300	2309	6	31.5	2.38	73	13.0
43-45	Apr. 5-16	75.5	99	90	300	2312	6	32.0	2.36	72	13.0
46-47	Apr. 17-24	75.1	75	90	300	2310	6	32.0	2.35	72	13.0

vegetable protein was studied. In Periods 1 to 4 protein was kept at approximately 25 grams and calories at 1750, while in the last 15 periods these values were 75 grams and 2310 calories respectively.

A similar procedure was followed in Case 2 (Table II). The first 16 periods were used to observe the effect of varying caloric intake from 1800 to 3770, with a fairly constant protein intake

of 75 grams, and the percentage of animal protein at 44 to 65 per cent. Periods 17 to 19 were "nitrogen free" periods for the purpose of studying the endogenous nitrogen metabolism. These were followed by two periods of fairly liberal protein intake, merely to fill up the depleted nitrogen stores somewhat before commencing the next series of studies. During the next 14 periods (22 to 35) the caloric intake was held at 3260 with

TABLE II  
Case 2. Composition of diets

Period number	Date	Protein	Animal protein	Fat	Carbohydrate	Calories	Salt	Average body weight	Protein per kgm.	Calories per kgm.	Calories from protein
		grams	per cent	grams	grams		grams	kgm.	grams		per cent
1-2	Sept. 30-Oct. 7	75	65	60	240	1800	3	51.1	1.47	35	16.7
3-4	Oct. 8-15	75	42	100	265	2260	5	50.9	1.47	44	13.3
5-6	Oct. 16-23	75	45	120	320	2660	5	51.4	1.46	52	11.3
7-8	Oct. 24-31	75	44	150	400	3250	5	52.3	1.43	62	9.2
9-10	Nov. 1-8	75	44	190	440	3770	5	53.0	1.42	71	8.0
11-12	Nov. 9-16	75	46	150	400	3250	5	54.0	1.39	60	9.2
13-14	Nov. 17-24	76	52	120	320	2664	5	53.7	1.42	50	11.4
15-16	Nov. 25-Dec. 2	75	62	100	265	2260	5	53.4	1.41	42	13.3
17-19	Dec. 3-14	2	0	105	330	2273	5	52.5	0.04	43	0.4
20-21	Dec. 15-22	75	40	100	265	2260	5	51.8	1.45	44	13.3
22-23	Dec. 23-30	75	50	150	400	3260	5	53.0	1.42	61	9.2
24-25	Dec. 31-Jan. 7	50	50	150	425	3260	5	53.7	0.93	61	6.1
26-27	Jan. 8-15	100	50	140	400	3260	5	54.7	1.82	60	12.2
28-30	Jan. 16-27	125	50	140	375	3260	5	55.6	2.25	59	15.3
31-33	Jan. 28-Feb. 8	150	50	130	373	3260	5	56.8	2.64	57	18.3
34-35	Feb. 9-16	175	50	125	359	3261	5	57.7	3.03	56	21.4
36-39	Feb. 17-Mar. 3	100	50	140	400	3260	5	58.5	1.71	56	12.3
40-42	Mar. 4-15	100	25	140	400	3260	5	59.3	1.69	55	12.3
43-47	Mar. 16-Apr. 4	100	0	140	400	3260	5	59.7	1.67	55	12.3
48-50	Apr. 5-16	100	98	140	400	3260	5	59.8	1.67	55	12.3
51-53	Apr. 17-28	100	75	140	400	3260	5	59.8	1.67	55	12.3

equal quantities of animal and vegetable protein, while the protein intake varied from 50 to 175 grams. In the last 18 periods (36 to 53), both the caloric and protein intake remained constant at 3260 and 100 grams respectively, while the proportion of animal protein was subjected to change from 0 to 98 per cent.

*Collection of urine and stools.* These were carried out as described in a previous study of nitrogen balance in nutritional edema (7).

*Analytical methods.* Urine nitrogen was determined on 24-hour collections by a modified Folin and Wright method (16). Proteins were separated from albumin-containing urine by adding an equal volume of 10 per cent trichloroacetic acid, and an aliquot of the filtrate was taken for non-protein nitrogen determination. Another untreated portion of urine was used for total nitrogen determination. The difference between the two determinations was taken to represent protein nitrogen. For stool nitrogen about 0.5 gram of dried stool, weighed on a chainomatic balance, was digested with 15 cc. of concentrated sulphuric acid, 6 cc. of 10 per cent ferric chloride, 10 drops of phosphoric acid and 5 drops of 5 per cent copper sulphate. Blood specimens were taken before breakfast with as little stasis as possible. Plasma proteins were separated by the method of Howe (17), and the albumin and globulin fractions de-

termined by the gasometric technique of Van Slyke (18).

## OBSERVATIONS AND COMMENTS

*Effect of caloric intake*

At a certain level of protein intake and with a given state of nutrition, on account of the protein-sparing action of carbohydrates and fat, the extent of positive nitrogen balance depends on the caloric intake. This is well illustrated in Case 2 (Table IV and Figure 2). This patient, while on a protein intake of 75 grams lost nitrogen on 1800 calories, or 35 calories per kgm., but gained nitrogen immediately when the caloric intake was increased to 2260, or 44 calories per kgm. Successive caloric additions increased the nitrogen retention until it reached a maximum of 3.44 grams daily at 3250 calories, or 62 calories per kgm. Further increase in caloric intake to 3770, however, did not improve the nitrogen balance. On decreasing the caloric intake, the positive nitrogen balance became progressively less.

In the first patient (Table III and Figure 1) the extent of positive nitrogen balance on 50 grams of protein was not significantly altered by successive additions of calories from 1750 to 2590, or from 65 to 88 calories per kgm. However, when the caloric intake was decreased from

TABLE III  
Case 1. *Effect of caloric intake.*  
*Protein 50 grams. Animal protein 50 per cent*

Period number	Calories	Average daily nitrogen metabolism						Body weight change	Plasma		
		Intake	Urine	Stool	Total	Balance	Average		Nonprotein nitrogen	Albumin	Total protein
		grams	grams	grams	grams	grams	grams	kgm.	mgm. per 100 cc.	grams per 100 cc.	grams per 100 cc.
5	1751	8.00	3.88	0.86	4.74	+3.26		+0.26	17	4.15	6.46
6	1751	8.00	4.33	1.26	5.59	+2.41	+2.78	+0.83	13	3.55	5.44
7	1751	8.00	4.09	1.23	5.32	+2.68		+0.31	20	3.39	5.66
8	2030	8.00	4.14	0.81	4.95	+3.05		+0.27	21	3.81	5.98
9	2030	8.00	4.31	0.82	5.13	+2.87	+2.96	+0.12	19	3.90	5.73
10	2311	8.00	4.51	0.94	5.45	+2.55		+0.50		3.95	5.81
11	2311	8.00	4.46	1.44	5.90	+2.10	+2.32	+0.48	17	3.33	5.65
12	2591	8.00	4.38	1.08	5.46	+2.54		+0.32	19	3.45	5.93
13	2591	7.96	4.58	1.15	5.73	+2.23	+2.38	+0.38	19	3.57	6.40
14	2310	8.00	5.72	1.27	6.99	+1.01		+0.31	22	3.70	6.06
15	2310	8.00	6.19	1.18	7.37	+0.63	+0.82	+0.34	22	3.81	6.16
16	2030	8.00	6.18	1.46	7.64	+0.36		-0.22		3.64	6.08
17	2030	8.00	6.47	1.05	7.52	+0.48	+0.42	+0.12	20	3.80	6.17
18	1750	8.00	6.60	1.30	7.90	+0.10		-0.16	22	3.59	5.76
19	1750	8.00	6.09	1.21	7.30	+0.70	+0.40	-0.13	19	3.65	5.74
20	1500	8.00	7.23	1.01	8.24	-0.24		-0.01		3.86	6.08
21	1500	8.00	6.85	0.94	7.79	+0.21	-0.02	-0.22	21	3.76	5.82



2590, he retained progressively less nitrogen so that when he was on 1500 calories, or 51 calories per kgm., he could hardly maintain nitrogen balance. As noted in the case report, this patient was convalescing from paratyphoid A fever before the studies were started. Consequently, his nitrogen stores were probably so impoverished that, from a diet of 50 grams of protein and 1750 calories, he was able to retain a relatively large amount of nitrogen. This amount was not in-

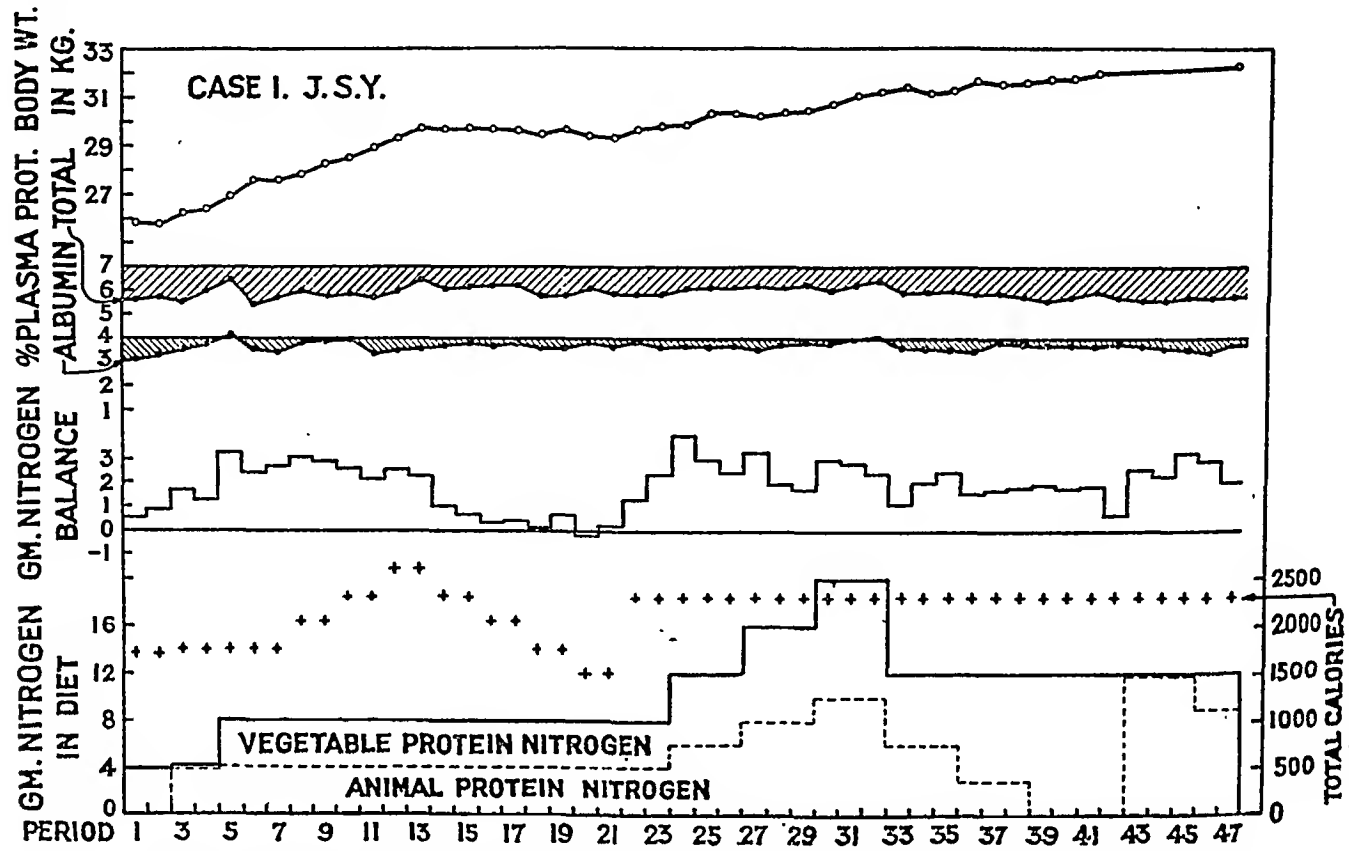


FIG. 1. CASE 1. NITROGEN BALANCE, PLASMA PROTEINS, AND BODY WEIGHT ON DIETS WITH VARYING TOTAL CALORIES, PROTEIN INTAKE, AND PROPORTION OF ANIMAL AND VEGETABLE PROTEIN.

TABLE IV  
Case 2. Effect of caloric intake  
Protein 75 grams. Animal protein 44 to 65 per cent

Period number	Calories	Average daily nitrogen metabolism							Body weight change kgm.	Plasma		
		Intake	Urine	Stool	Total output	Balance	Average balance	Urine protein nitrogen		Nonprotein nitrogen	Albumin	Total protein
		grams	grams	grams	grams	grams	grams	grams		mgm. per 100 cc.	grams per 100 cc.	grams per 100 cc.
1	1800	12.00	12.19	1.39	13.58	-1.58	-1.63	2.00	-0.58	29	2.27	3.96
2	1800	12.00	12.31	1.38	13.69	-1.68	-1.63	1.59	-0.23	30	2.14	4.20
3	2260	12.00	9.97	1.50	11.47	+0.53	+1.08	1.31	+0.18	36	2.19	4.45
4	2260	12.00	8.72	1.66	10.38	+1.62	+1.08	1.22	+0.19	24	2.62	4.91
5	2660	12.00	7.79	1.73	9.52	+2.48	+2.65	1.22	+0.44		2.51	4.75
6	2660	12.00	7.18	2.00	9.18	+2.82	+2.65	1.21	+0.40	20	2.72	4.94
7	3250	12.00	6.82	1.63	8.45	+3.55	+3.44	1.26	+0.63	18	2.68	4.90
8	3250	12.00	6.92	1.76	8.68	+3.32	+3.44	1.40	+0.07		2.87	5.08
9	3770	12.00	6.78	2.13	8.91	+3.09	+3.13	1.40	+0.39	16	2.91	5.20
10	3770	12.00	6.84	1.99	8.83	+3.17	+3.13	1.70	+0.98	18	3.11	5.33
11	3250	12.00	7.40	2.02	9.42	+2.58	+2.74	1.45	+0.08	16	2.76	4.66
12	3250	12.00	7.48	1.63	9.11	+2.89	+2.74	1.28	+0.04	23	2.80	4.93
13	2664	12.00	8.68	1.63	10.31	+1.69	+1.51	1.26	-0.10	24	3.12	5.19
14	2664	12.00	9.02	1.53	10.57	+1.43	+1.51	1.00	-0.50	22	3.03	5.28
15	2294	13.36	10.48	1.39	11.87	+1.49		1.06	-0.10		3.06	5.11
16	2260	12.00	10.42	1.46	11.88	+0.12		1.30	+0.10	20	3.08	5.14

creased by subsequent caloric additions as his nitrogen hunger, so to speak, was gradually satisfied. With nitrogen stores relatively replete in the latter part of the study, a caloric intake less than 2591, or 88 per kgm., did not insure a maximum nitrogen balance. Such a high caloric diet, however, could not be maintained for a long time. For that reason only 2310 calories were given subsequently, when the effects of protein intake were studied, and this level of caloric intake was considered the optimum.

the amount of urinary protein nitrogen did not vary regularly or significantly, although it showed a perceptible tendency to decrease with improvement of the patient's general condition.

In the first patient plasma albumin remained approximately at 3.7 per cent and total protein at 5.9 per cent, values above the lower limits of normal. In the second patient there was a steady increase in albumin as well as in total protein as the caloric level of the diet was raised, although subsequent lowering of the caloric intake failed

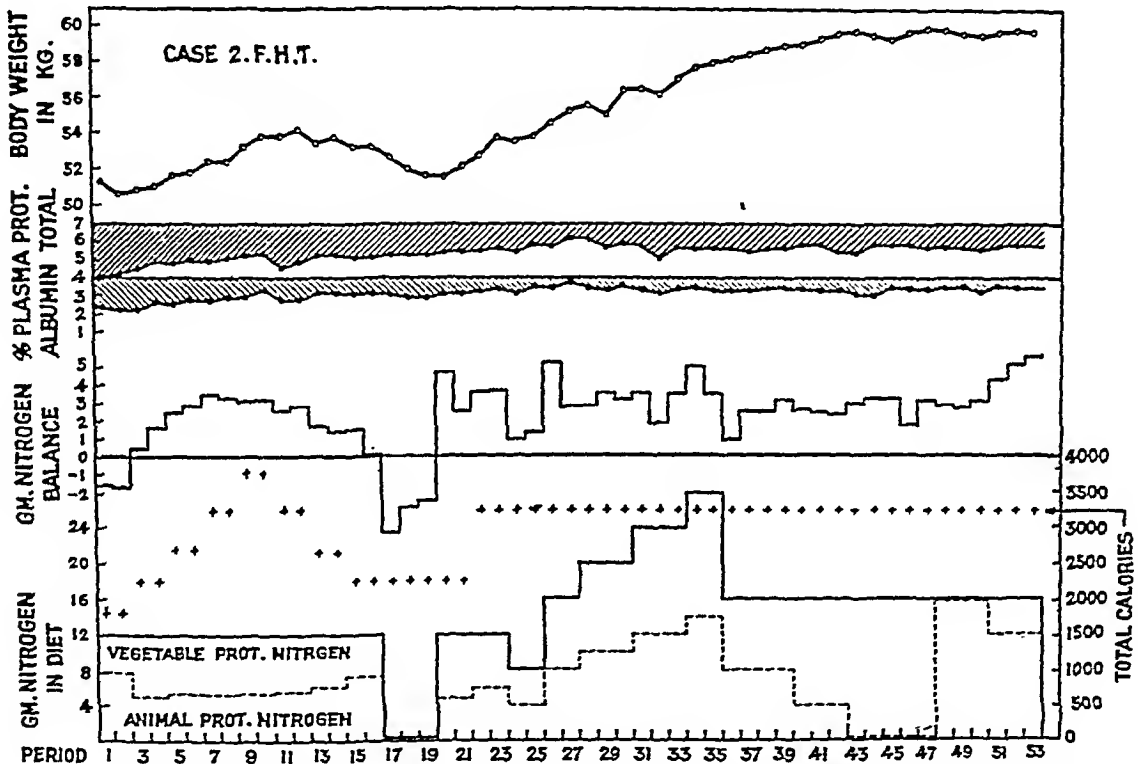


FIG. 2. CASE 2. NITROGEN BALANCE, PLASMA PROTEINS, AND BODY WEIGHT ON DIETS WITH VARYING TOTAL CALORIES, PROTEIN INTAKE, AND PROPORTION OF ANIMAL AND VEGETABLE PROTEIN.

Both patients gained weight with higher caloric diets. As the first patient had no edema, and the second patient showed at the beginning of the study only slight pitting edema over the tibiae which disappeared completely a little later, it is assumed that the weight gained represented body tissue. The tendency to gain weight, like the positive nitrogen balance, became less as time went on, so that a higher caloric intake was necessary to bring about a gain in weight in the latter part of the experiment than in the first studies.

In Case 2, in which albuminuria was present,

to bring about a reverse change in protein concentration. The plasma albumin rose from 2.27 to 3.08 per cent and total protein from 3.96 to 5.14 per cent within the period of two months' study corresponding to the transition from slight pitting edema to complete disappearance of edema, a point of interest in connection with the pathogenesis of nephrotic edema.

#### *Effect of different levels of protein intake*

With a constant caloric intake, the degree of nitrogen gain can be increased by raising the

TABLE V

Case 1. Effect of different levels of protein intake.  
Calories 2310. Animal protein 50 per cent

Period number	Protein	Average daily nitrogen metabolism						Body weight change	Plasma		
		Intake	Urine	Stool	Total	Balance	Average		Nonprotein nitrogen	Albumin	Total protein
	grams	grams	grams	grams	grams	grams	grams	kgm.	mgm. per 100 cc.	grams per 100 cc.	grams per 100 cc.
22	50	8.00	5.35	1.32	6.67	+1.33	+1.86	+0.43	17	3.86	5.78
23	50	8.00	4.02	1.60	5.62	+2.38		+0.19	24	3.65	5.83
24	75	12.00	6.45	1.57	8.02	+3.98		+0.14	24	3.72	6.10
25	75	12.00	7.50	1.53	9.03	+2.97	+3.11	+0.36	23	3.68	6.13
26	75	12.00	8.21	1.40	9.61	+2.39		+0.25	19	3.74	6.12
27	100	16.00	10.96	1.72	12.68	+3.32		-0.26	25	3.57	6.18
28	100	16.00	13.28	0.74	14.02	+1.98	+2.35	-0.03	25	3.78	6.09
29	100	16.00	12.75	1.51	14.26	+1.74		+0.14	27	3.90	6.25
30	125	20.00	15.01	1.99	17.00	+3.00		+0.29	17	3.82	6.01
31	125	20.00	15.15	2.02	17.17	+2.83	+2.75	+0.11	21	4.01	6.27
32	125	20.00	15.48	2.11	17.59	+2.41		+0.26	24	4.11	6.44

level of protein feeding to a certain extent, beyond which further increase in protein intake no longer improves the nitrogen balance, even if patients can tolerate the high protein diet.

In Case 1 (Table V), with a constant intake of 2310 calories, a maximum nitrogen retention of 3.11 grams daily was reached, the diet containing 75 grams of protein, or 2.5 grams per kgm. In Case 2 (Table VI), with a constant caloric in-

take of 3250, a maximum of 4.02 grams of nitrogen per day was retained when 100 grams of protein, or 1.8 gram per kgm., were fed. These amounts of protein correspond to 12 to 13 per cent of the total caloric intake. Further increase in protein failed to increase the nitrogen retention. In fact, nitrogen balances with protein intakes higher than 12 to 13 per cent were slightly lower, except during Periods 34 and 35 in Case 2, in

TABLE VI

Case 2. Effect of the variations in protein intake.

Calories: 2270 for Periods 17 to 21 and 3260 for Periods 22 to 35. Animal protein: none for Periods 17 to 19, 40 per cent for Periods 20 and 21, and 50 per cent for the remaining periods

Period number	Protein	Average daily nitrogen metabolism							Body weight change	Plasma		
		Intake	Urine	Stool	Total	Balance	Average balance	Urine protein nitrogen		Nonprotein nitrogen	Albumin	Total protein
	grams	grams	grams	grams	grams	grams	grams	grams	kgm.	mgm. per 100 cc.	grams per 100 cc.	grams per 100 cc.
17	2	0.33	3.79	0.82	4.61	-4.28	-3.20	0.81	-0.70	17	3.07	5.27
18	2	0.37	2.56	0.66	3.22	-2.85		0.58	-0.91	16	2.96	5.34
19	2	0.37	2.19	0.64	2.83	-2.46		0.49	-0.14	16	2.89	5.35
20	75	12.00	6.01	1.25	7.26	+4.73	+3.64	0.57	+0.13	30	3.06	5.41
21	75	12.00	7.56	1.91	9.47	+2.53		0.68	+0.19	27	3.03	5.43
22	75	12.00	6.41	1.94	8.35	+3.65		0.66	+1.34	24	3.21	5.44
23	75	12.00	6.44	1.82	8.26	+3.74	+1.18	0.59	+0.61	20	3.28	5.62
24	50	8.00	5.43	1.60	7.03	+0.97		0.51	-0.98	18	3.05	5.38
25	50	8.00	4.98	1.64	6.62	+1.38		0.58	+0.96	19	3.43	5.78
26	100	16.00	8.63	2.11	10.74	+5.26	+4.02	0.50	+0.90	29	3.45	5.77
27	100	16.00	10.88	2.33	13.21	+2.79		0.39	+0.44	29	3.70	6.22
28	125	19.02	13.50	2.71	16.21	+2.81		0.51	+0.15	30	3.44	6.11
29	125	20.00	14.16	2.26	16.42	+3.58	+3.19	0.50	+0.71	32	3.32	5.68
30	125	20.00	14.48	2.34	16.82	+3.18		0.76	+0.40	32	3.49	5.94
31	150	24.00	17.48	2.97	20.45	+3.55		0.69	+0.16	29	3.35	5.77
32	150	24.00	19.13	3.01	22.14	+1.86	+2.97	0.75	+0.34	32	3.11	5.06
33	150	24.00	17.72	2.78	20.50	+3.50		0.73	+0.32	32	3.33	5.70
34	175	27.80	19.51	3.24	22.75	+5.05		0.59	+0.54	35	3.39	5.61
35	175	25.18	18.54	3.08	21.62	+3.56	+4.30	0.42	+0.04	36	3.24	5.39

which protein feeding to the level of 21.4 per cent caused a nitrogen gain of 4.30 grams per day. However, such a high level of protein intake was not tolerated by the patient for more than a few days.

It is to be noted that the first patient required more protein, as well as more calories per kgm., than the second patient, to produce a maximum nitrogen retention. This may be referable to the age of the first patient, which was only 13, a time when active growth goes on. In the case of nephrosis in a child of six reported by Cowie et al. (19), a satisfactory nitrogen gain was attained by feeding 88 calories and 3.8 grams of protein per kgm.

Case 2 afforded an opportunity to observe the effects of an almost protein-free diet. This was given for 12 days (Periods 17, 18, and 19). There was a marked negative nitrogen balance with loss of body weight. However, nitrogen loss tended to diminish so that only 2.46 grams of nitrogen were lost daily during the last period. The average nitrogen excretion amounted to 2.83 grams per day. When stool and urinary protein nitrogen are subtracted from the total excretion, the urinary nonprotein nitrogen amounts to 1.70 gram. This figure represents 0.033 gram of nitrogen per kgm. of body weight, a nitrogen minimum that is comparable with the results obtained in normal individuals (20). The two following periods (20 and 21) with the same caloric intake, but with 75 grams of protein, were intended to make up the nitrogen loss of the preceding periods on a "nitrogen-free" diet, preliminary to the studies of the effect of different levels of protein intake, reported above. Incidentally, the results of these two periods demonstrate the great avidity with which nitrogen is retained after periods of nitrogen depletion. The nitrogen gain during these two periods is the same as that in the next two periods (22 and 23) in which the energy of the diet was increased by approximately 1000 calories and is much better than the results of Periods 3 and 4 (Table IV) with the same caloric and protein intake.

In Case 2, the proteinuria did not change significantly with the changes in the level of dietary protein.

Plasma proteins tended to increase slightly with progressive increases of protein intake in Case 1,

but did not change significantly in Case 2 when various levels of protein were fed. Plasma non-protein nitrogen remained fairly constant throughout in Case 1, but to some extent varied directly with the level of protein feeding in Case 2.

#### *Comparison between animal and vegetable protein*

In Case 1 (Table VII) comparison of the effect on nitrogen balance of varying the proportions of animal and vegetable protein was made at two different times with different levels of protein and caloric intake. In Periods 1 to 4, with protein at 25 grams and calories at 1750, the nitrogen balance obtained with animal protein was decidedly superior to that with vegetable protein. During Periods 33 to 47, with protein at 75 grams and calories at 2310, variations in the percentage of animal protein between 0 and 99 per cent resulted in slight changes in nitrogen balance. There was a small increase of nitrogen retention when the proportion of animal protein was progressively raised. In Case 2 (Table VIII) no clear-cut differences in nitrogen balance attended variation of the proportion of animal protein, although the nitrogen gain was greater during the three periods (51 to 53) on 75 per cent of animal protein than in any other periods. When optimum protein and caloric intake was maintained, the relative proportions of animal and vegetable protein in the diet did not seem to have much influence on nitrogen retention.

The albuminuria in Case 2 again showed no marked variation, although a tendency to decrease was discernible during the course of study.

No marked variations were observed in either case in plasma protein or nonprotein nitrogen when the proportions of animal and vegetable protein were varied.

#### SUMMARY AND CONCLUSIONS

1. In two patients with the nephrotic type of Bright's disease, one aged 13 and the other aged 35, nitrogen balances and plasma proteins were studied in relation to diets in which total calories, protein content, and percentage of animal and vegetable protein were varied.

2. With a moderate protein intake, nitrogen retention could be progressively increased by the addition of calories in the form of carbohydrate

and fat. The first patient showed a maximum nitrogen gain at 88 calories per kgm., and the second patient retained the greatest amount of nitrogen at 62 calories per kgm. Further increase in caloric intake resulted in no increases in the nitrogen retained.

3. When these patients were fed the optimum number of calories, raising the protein intake increased the nitrogen retention somewhat. The maximum nitrogen retention was reached when 75 grams of protein were fed in Case 1 and with 100 grams in Case 2. These amounts correspond

TABLE VII

*Case 1. Effect of different proportions of animal and vegetable protein.  
Calories 1750 and protein 25 grams for Periods 1 to 4. Calories 2310 and proteins 75 grams for Periods 33 to 47*

Period number	Animal protein	Average daily nitrogen metabolism						Body weight change	Plasma		
		Intake	Urine	Stool	Total	Balance	Average		Nonprotein nitrogen	Albumin	Total protein
	<i>per cent</i>	<i>grams</i>	<i>grams</i>	<i>grams</i>	<i>grams</i>	<i>grams</i>	<i>grams</i>	<i>kgm.</i>	<i>mgm. per 100 cc.</i>	<i>grams per 100 cc.</i>	<i>grams per 100 cc.</i>
1	0	3.95	2.13	1.27	3.40	+0.55	+0.72	+0.31		3.12	5.61
2	0	3.95	2.20	0.87	3.07	+0.88		-0.06	15	3.26	5.64
3	92	4.04	1.78	0.55	2.33	+1.71	+1.47	+0.40		3.54	5.51
4	92	4.04	1.91	0.90	2.81	+1.23		+0.10	15	3.74	5.99
33	50	11.97	9.72	1.15	10.87	+1.10		-0.09	19	3.65	5.94
34	50	11.97	8.76	1.15	9.91	+2.06	+1.86	+0.02	30	3.60	5.94
35	50	11.97	8.72	0.82	9.54	+2.43		+0.11	18	3.55	5.96
36	25	12.03	8.21	2.20	10.41	+1.62		+0.25	20	3.51	5.84
37	25	12.03	8.75	1.54	10.29	+1.74	+1.72	-0.05	21	3.85	5.87
38	25	12.03	8.38	1.86	10.24	+1.79		+0.39	21	3.73	5.78
39	0	11.97	7.54	2.52	10.06	+1.91		-0.07	19	3.67	5.49
40	0	11.97	7.77	2.35	10.12	+1.85	+1.58	-0.01	19	3.73	5.72
41	0	11.97	7.80	2.25	10.05	+1.92		+0.23	20	3.67	5.97
42	0	11.97	8.44	2.90	11.34	+0.63			22	3.77	5.70
43	99	12.08	8.40	1.08	9.48	+2.60			19	3.62	5.81
44	99	12.08	8.84	0.92	9.76	+2.32	+2.74		20	3.60	5.62
45	99	12.08	7.97	0.82	8.79	+3.29			21	3.54	5.77
46	75	12.02	8.12	0.94	9.06	+2.96	+2.48		20	3.40	5.72
47	75	12.02	9.04	0.97	10.01	+2.01			22	3.71	5.76

TABLE VIII

*Case 2. Variations in percentage of animal and vegetable proteins.  
Calories 3260. Protein 100 grams*

Period number	Animal protein	Average daily nitrogen metabolism							Body weight change	Plasma		
		Intake	Urine	Stool	Total	Balance	Average	Urine protein N <sub>2</sub>		Nonprotein nitrogen	Albumin	Total protein
	<i>per cent</i>	<i>grams</i>	<i>grams</i>	<i>grams</i>	<i>grams</i>	<i>grams</i>	<i>grams</i>	<i>grams</i>	<i>kgm.</i>	<i>mgm. per 100 cc.</i>	<i>grams per 100 cc.</i>	<i>grams per 100 cc.</i>
36	50	15.96	13.20	1.82	15.02	+0.94		0.76	+0.55	25	3.27	5.65
37	50	15.96	11.88	1.51	13.39	+2.57	+2.33	0.63	-0.16		3.41	5.59
38	50	15.96	11.32	2.06	13.38	+2.58		0.72	+0.68	26	3.28	5.41
39	50	15.96	11.02	1.72	12.74	+3.22		0.56	+0.08	31	3.36	5.61
40	25	15.98	11.20	2.14	13.34	+2.64		0.53	+0.23	27	3.42	5.59
41	25	15.98	11.46	2.04	13.50	+2.48	+2.52	0.16	+0.17	28	3.32	5.87
42	25	15.98	11.64	1.90	13.54	+2.44		0.29	-0.06	30	3.27	5.86
43	0	15.97	10.98	2.05	13.03	+2.95		0.63	+0.36	30	3.25	5.42
44	0	15.97	10.63	2.09	12.72	+3.25		0.34	-0.20	26	3.09	5.40
45	0	15.97	10.72	2.01	12.73	+3.25	+2.88	0.51	-0.22	24	3.48	5.86
46	0	15.97	12.11	2.05	14.16	+1.81		0.57	+0.56	25	3.42	5.86
47	0	15.97	10.66	2.19	12.85	+3.12		0.31	+0.23	27	3.39	5.79
48	98	16.02	11.94	1.18	13.12	+2.90		0.41	-0.10	30	3.48	5.72
49	98	16.02	12.37	0.88	13.25	+2.77	+2.93	0.38	-0.18	30	3.46	5.72
50	98	16.02	11.98	0.91	12.89	+3.13		0.30	-0.01	30	3.13	5.52
51	75	15.94	10.18	1.37	11.55	+4.39		0.24	+0.52	24	3.48	5.82
52	75	15.94	9.75	0.99	10.74	+5.20	+5.05	0.18	-0.29	29	3.38	5.78
53	75	15.94	8.88	1.46	10.34	+5.60		0.18	-0.37			

to 2.5 grams and 1.8 gram per kgm. of body weight and constitute 12 to 13 per cent of the total calories. Feeding more protein did not increase the positive nitrogen balance.

4. The first patient showed a slightly increasing nitrogen retention as higher percentages of animal protein were fed, while in the second patient the nature of protein made no distinct difference. While it is impractical to feed a purely animal protein diet, a diet with at least 50 per cent of its protein of animal origin would seem to insure the best nitrogen gain.

5. Changes in plasma proteins were slow, even in the presence of marked nitrogen retention. The proteins remained at about the same level throughout Case 1. In Case 2 they slowly increased during the early part of the study.

6. In the second patient, in whom albuminuria decreased as the study progressed, there was no significant change in the albuminuria related to the level of protein intake.

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# THE EFFECT OF VENESECTION ON ARTERIAL, SPINAL FLUID, AND VENOUS PRESSURES WITH ESPECIAL REFERENCE TO FAILURE OF THE LEFT AND RIGHT HEART

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(From the Department of Medicine, University of Pennsylvania Medical School, Philadelphia)

(Received for publication September 13, 1934)

The correlation of venous, spinal fluid, and arterial pressure, before and after venesection, was studied in a series of twenty-two cases of failure of the left heart and thirteen cases of failure of the right heart. These cases were observed clinically with reference to the improvement after venesection alone, and also after venesection in combination with the removal of twenty cubic centimeters of spinal fluid.

The cases studied embraced various types of heart disease, including syphilitic, rheumatic, arteriosclerotic, and hypertensive.

## LITERATURE

Eyster (1) attributed the value of venesection in cardiac congestion to the reduction of a high venous blood volume. He contended that by this procedure the right ventricle is relieved of an abnormal initial strain.

Harrison (2) in 1933 made the observation that cisternal pressure was lower in the upright than in the prone position, whereas venous pressure was higher in the upright than in the prone position. He found also that venous, spinal fluid, and cisternal pressures were all more elevated in patients with congestive heart failure than in patients with normal circulation.

Harrison (3) in 1934 concluded from his studies on cisternal pressure that the patient with cardiac congestion secures more relief from his orthopnea when in the sitting position because of lowered cisternal pressure, and in turn lessened embarrassment to the respiratory center. Spinal drainage, in most cases, was followed by a decrease in dyspnea.

Friedfeld and Fishberg (4) in their studies of "The Relation of the Cerebrospinal and Venous Pressures in Heart Failure" showed that an elevation of venous pressure in failure of the right heart was invariably associated with an elevation of the spinal fluid pressure. They noted that venous and cerebrospinal pressures were lowered

as cardiac compensation was restored. An elevation of the spinal fluid pressure did not occur in failure of the left heart with a normal venous pressure.

Gravier (5) found no definite correlation between arterial and spinal fluid pressures.

Tzanck and Renault (6), Eyster and Middleton (7), and Clark (8), in clinical observations, found a close correlation between the deep venous (internal jugular) and spinal fluid pressures. These authors selected 200 mm. of water as the critical level of venous pressure at which venesection is indicated.

Myerson and Loman (9), as well as Planques, Riser, and Sorel (10) found that arterial hypertension alone would not induce elevation of pressure in the cerebrospinal fluid, but that pressure of the latter was dependent upon the venous pressure.

Additional studies of venous and spinal fluid pressures not so directly related to our problem are listed in the bibliography (11-16).

## METHODS

In failure of the right heart, we include those cases manifesting signs of venous congestion, dyspnea, cyanosis, ascending edema, effusion into the serous sacs, and enlargement of the liver.

In failure of the left heart, we include those cases with cardiac dyspnea without engorgement of the systemic veins and also cases of diminished vital capacity with cardiac asthma. The presence of protodiastolic gallop rhythm or accentuation of the pulmonic second sound inclined us to classify a case as in this group. There was usually, in these cases, no enlargement of the liver, no cyanosis, and no venous congestion.

Normal values for spinal fluid pressure taken between the 3d and 4th lumbar vertebrae in the erect position we consider as varying from 150 to 400 mm. of water.

Normal values for venous pressure at the level





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Normal values for spinal fluid pressure taken between the 3d and 4th lumbar vertebrae in the erect position we consider as varying from 150 to 400 mm. of water.

Normal values for venous pressure at the level

of the right auricle taken in the upright position we take as from 90 to 125 mm. of water.

All readings were taken in the morning with the patients in the sitting position. The lumbar puncture needle was inserted in the usual manner, and a mercury manometer used to determine pressure. The mercury pressure was later calculated and expressed in millimeters of water. The ar-

terial and venous pressure readings were then recorded, the former with a mercury sphygmomanometer, and the latter with an L glass tube of 7 mm. diameter inserted into the antecubital vein. Care was exercised in reading the venous pressure to have the antecubital vein precisely at the level of the right auricle. When these three readings had been made, the patients were bled eight to

TABLE I

*Arterial, cerebrospinal fluid, and venous pressures before and after venesection in failure of the right heart*

Patient number	Arterial pressure		Cerebrospinal fluid pressure			Venous pressure		
	Before	After	Before	After	Change	Before	After	Change
	<i>mm. Hg</i>	<i>mm. Hg</i>	<i>mm. H<sub>2</sub>O</i>	<i>mm. H<sub>2</sub>O</i>	<i>mm. H<sub>2</sub>O</i>	<i>mm. H<sub>2</sub>O</i>	<i>mm. H<sub>2</sub>O</i>	<i>mm. H<sub>2</sub>O</i>
1.....	190/140	175/125	408	272	-136	245	95	-150
2.....	60/10	64/40	354	272	-82	160	135	-25
3.....	135/105	140/100	462	326	-136	220	105	-115
4.....	146/118	150/120	408	354	-54	140	50	-90
5.....	172/124	162/110	435	367	-68	195	80	-115
6.....	190/130	190/130	394	394	0	195	120	-75
7.....	194/144	194/144	408	272	-136	200	108	-92
8.....	220/160	210/160	408	204	-204	190	130	-60
9.....	180/80	165/70	490	381	-109	265	140	-125
10.....	180/70	164/66	435	326	-109	160	100	-60
11.....	265/150	265/145	490	381	-109	220	135	-85
12.....	180/140	168/135	435	408	-27	165	140	-25
13.....	175/150	175/140	462	381	-81	230	160	-70
Average.....			430	334	-96	199	115	-84

TABLE II

*Arterial, cerebrospinal fluid, and venous pressures before and after venesection in failure of the left heart*

Patient number	Arterial pressure		Cerebrospinal fluid pressure			Venous pressure		
	Before	After	Before	After	Change	Before	After	Change
	<i>mm. Hg</i>	<i>mm. Hg</i>	<i>mm. H<sub>2</sub>O</i>	<i>mm. H<sub>2</sub>O</i>	<i>mm. H<sub>2</sub>O</i>	<i>mm. H<sub>2</sub>O</i>	<i>mm. H<sub>2</sub>O</i>	<i>mm. H<sub>2</sub>O</i>
1.....	202/114	206/116	490	462	-28	90	70	-20
2.....	225/20	180/50	326	326	0	72	58	-14
3.....	198/102	180/94	299	299	0	110	95	-15
4.....	228/78	204/75	163	136	-27	95	85	-10
5.....	150/20	140/20	476	449	-27	70	65	-5
6.....	264/158	225/150	490	422	-68	80	80	0
7.....	198/156	198/152	326	381	+55	70	70	0
8.....	162/54	160/72	326	326	0	135	90	-45
9.....	182/112	182/110	299	313	+14	100	50	-50
10.....	184/66	120/98	490	408	-82	124	95	-29
11.....	180/100	170/86	354	272	-82	85	75	-10
12.....	160/130	160/130	340	340	0	50	80	+30
13.....	280/160	300/170	326	381	+55	30	85	+55
14.....	180/104	150/112	367	367	0	120	110	-10
15.....	250/170	230/170	408	408	0	90	90	0
16.....	142/94	142/94	408	408	0	90	90	0
17.....	230/125	230/125	462	381	-81	125	115	-10
18.....	148/118	138/110	299	272	-27	100	80	-20
19.....	120/80	155/90	354	326	-28	70	25	-45
20.....	170/110	170/110	299	299	0	95	90	-5
21.....	138/90	126/84	326	326	0	135	125	-10
22.....	178/126	162/104	381	326	-55	135	118	-17
Average.....			364	347	-17	94	84	-10

twelve ounces. Subsequent to the venesection the same observations were again noted. Adequate time was allowed before each reading so that the patients' excitement would subside and not produce error. The sitting position was chosen because the readings were more accurate and no change of posture was necessary after the observations were begun.

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#### OBSERVATIONS

##### *Failure of the right heart*

A close relationship was found to exist between the spinal fluid and venous pressure in right heart failure. Both pressures were elevated in this type of congestive failure; the spinal fluid tension was always higher than the venous pressure, and a definite parallelism was observed. Arterial blood pressure had no relation whatever to the other two pressures. Following venesection of eight to twelve ounces, the venous and spinal fluid pressures consistently fell. Table I depicts the arterial, spinal fluid, and venous pressures before and after venesection. It is apparent from this table that spinal fluid pressure was consistently higher than venous pressure and that both were considerably above normal. The average spinal fluid pressure in millimeters of water before venesection was 430, and after venesection, 334. The average fall of lumbar spinal fluid pressure was 96. In only one of thirteen cases was no change noted. The average venous pressure before venesection was 199, and after venesection 115. The average fall of venous pressure was 84. The ratio of the mean spinal fluid pressure to mean venous pressure was not significantly altered by venesection (see Table III). Before venesection, it was 2.16; after venesection, 2.90. Venous pressure fell in all cases. The arterial pressure fell in six cases, rose in three, and remained approximately the same in four. Orthopnea was lessened in all patients immediately after venesection. In eight of the thirteen subjects, ten to twenty cubic centimeters of spinal fluid was withdrawn. We found that those patients who received both lum-

bar drainage and venesection obtained the most rapid and permanent relief from their orthopnea.

##### *Failure of the left heart*

The close relation which existed between venous and spinal fluid pressures in right myocardial failure did not obtain in left heart failure. Table II shows the lower venous and spinal fluid pressures in the latter condition.

Inspection of Figure 3 reveals seven instances in which the spinal pressures are elevated above the normal values. This is attributed to the hydrostatic pressure effect, since these patients were elevated from a recumbent to a sitting position in order to determine the measurements. The remainder were initially in the upright position.

TABLE III

*Ratios of mean spinal fluid and mean venous pressures before and after venesection*

Ratios	Right heart failure	Left heart failure
1. Ratio of the mean fall of spinal fluid pressure to mean fall of venous pressure.....	1.14	1.70
2. Ratio of the mean spinal fluid pressure to mean venous pressure before venesection.....	2.16	3.77
3. Ratio of the mean spinal fluid pressure to mean venous pressure after venesection.....	2.90	4.13

The average venous pressures before and after venesection in Table I are 199 and 115, whereas in Table II they are 94 and 84. The average fall of venous pressure after bleeding was 84 in the right heart cases and 10 in the left. The average spinal fluid pressure in failure of the left heart was, before venesection, 364, and after venesection, 347, with an average fall of 17. The ratios of the mean values are given in Table III. Venous pressure fell in sixteen cases, rose in two, and remained constant in four. Arterial pressure again showed no relation to the spinal fluid and venous pressures. It rose in four cases, fell in twelve and remained the same in six. The presence of arterial hypertension did not indicate existing venous hypertension. The spinal fluid pressure fell in ten cases, rose in three, and remained unchanged in nine.

We feel justified in stating that venesection in hypertensive cardiovascular disease is not to be recommended unless there is elevation of the venous pressure, suggesting right ventricular failure.

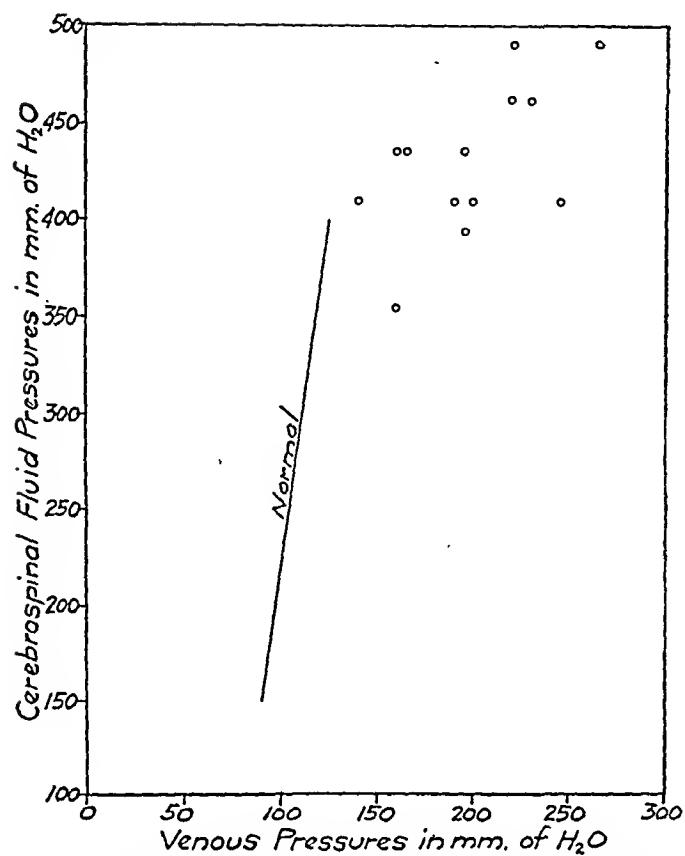


FIG. 1. CEREBROSPINAL FLUID PRESSURES AND VENOUS PRESSURES IN RIGHT HEART FAILURE. (Before venesection.)

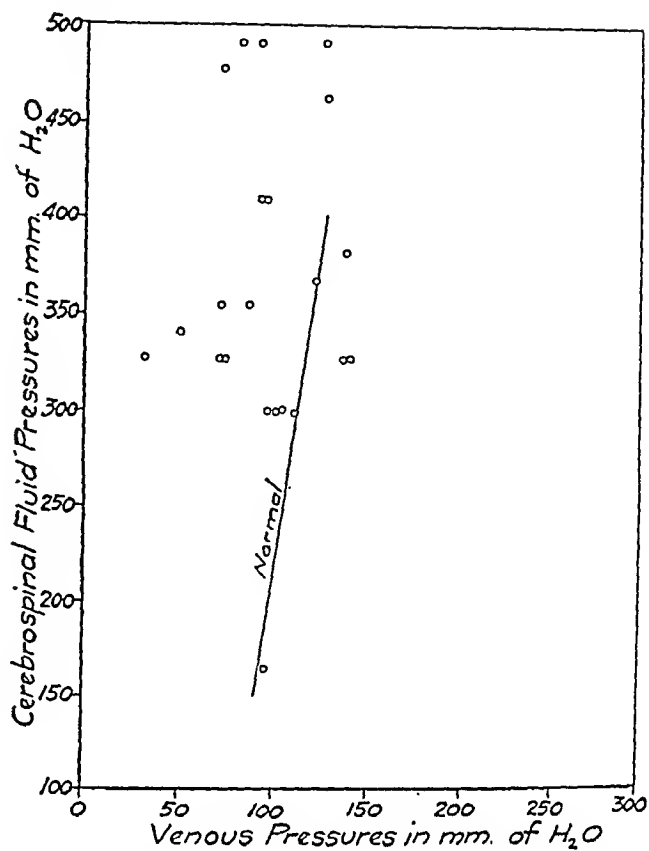


FIG. 3. CEREBROSPINAL FLUID PRESSURES AND VENOUS PRESSURES IN LEFT HEART FAILURE. (Before venesection.)

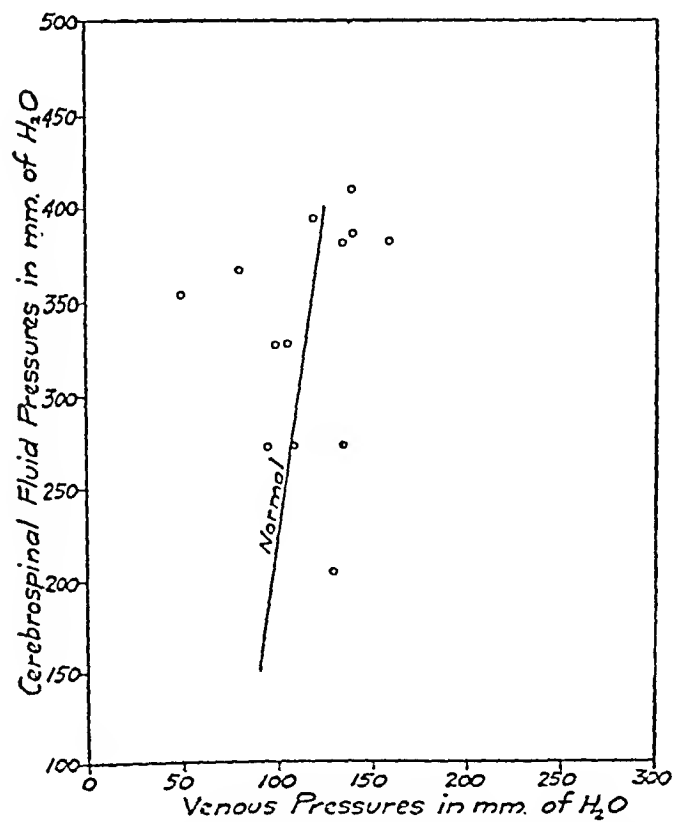


FIG. 2. CEREBROSPINAL FLUID PRESSURES AND VENOUS PRESSURES IN RIGHT HEART FAILURE. (After venesection.)

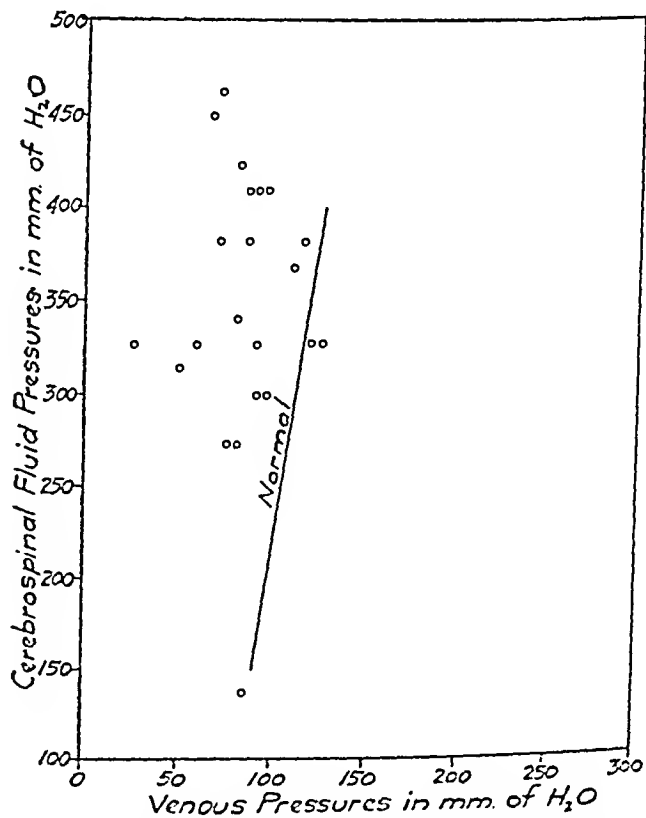


FIG. 4. CEREBROSPINAL FLUID PRESSURES AND VENOUS PRESSURES IN LEFT HEART FAILURE. (After venesection.)

## DISCUSSION

The foregoing observations show that venous and spinal fluid pressures are elevated in failure of the right heart, and that both fall after venesection. Inspection of Figures 1 and 2 reveals a tendency to parallel variation. Variation in the ratio between the two variables is shown by the divergences from a strictly linear distribution.

Since, in right heart failure, the demand upon the heart exceeds its physiologic response, it cannot convey into the pulmonary circulation the blood which comes to it from the venous circulation. Venous engorgement and stasis result consequently, especially upon failure of the right ventricle. It has long been known that phlebotomy relieves such venous and ventricular embarrassment.

We believe that if the pressure on the respiratory center is lessened by spinal fluid drainage the patient will obtain greater benefit than from venesection alone. With this in view, we combine the two procedures and find ample justification in the immediate clinical response.

Since, in failure of the left heart, the venous pressure is rarely or never elevated, treatment by combined venesection and lumbar drainage meets with little success. This method is found to be of no value in the hypertensive subject with a yielding of the left myocardium. Previous investigators have shown that arterial hypertension alone does not elevate spinal fluid pressure, but that the latter is dependent upon the venous tension. Our studies indicate no relation whatsoever between the arterial and the venous or spinal fluid pressures.

A thorough review of the literature revealed no similar study of the correlation between the three pressures. We think also that the combination of venesection with spinal drainage is an innovation in the treatment of failure of the right heart.

## SUMMARY

A series of experiments was carried out to determine the relation of arterial, spinal fluid, and venous pressures before and after venesection.

It was found in right heart failure that venous and spinal fluid pressures were elevated and re-

lated with respect to fall of pressures induced by venesection. The variation in the ratio between the two pressures is shown by the divergences from a linear distribution.

The spinal fluid pressure was elevated above normal in 32 per cent and 85 per cent of left and right heart failures, respectively. No correlation obtained between the arterial blood pressure and the venous or spinal fluid pressures in either right or left cardiac incompetence. The venous and spinal fluid pressures were uncorrelated in failure of the left heart. The spinal pressure was greater than the venous pressure in all of 140 observations made on 35 patients.

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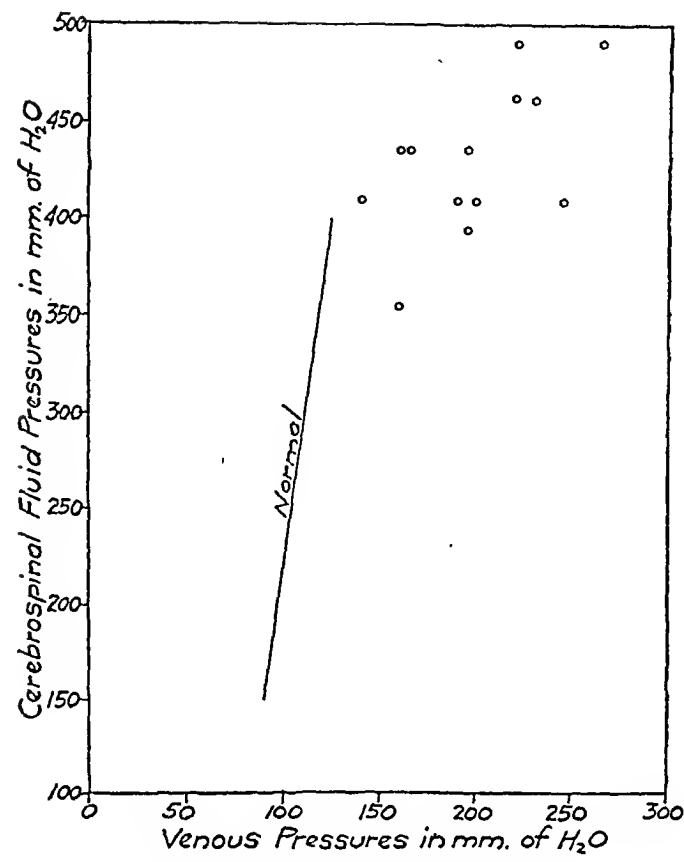


FIG. 1. CEREBROSPINAL FLUID PRESSURES AND VENOUS PRESSURES IN RIGHT HEART FAILURE. (Before venesection.)

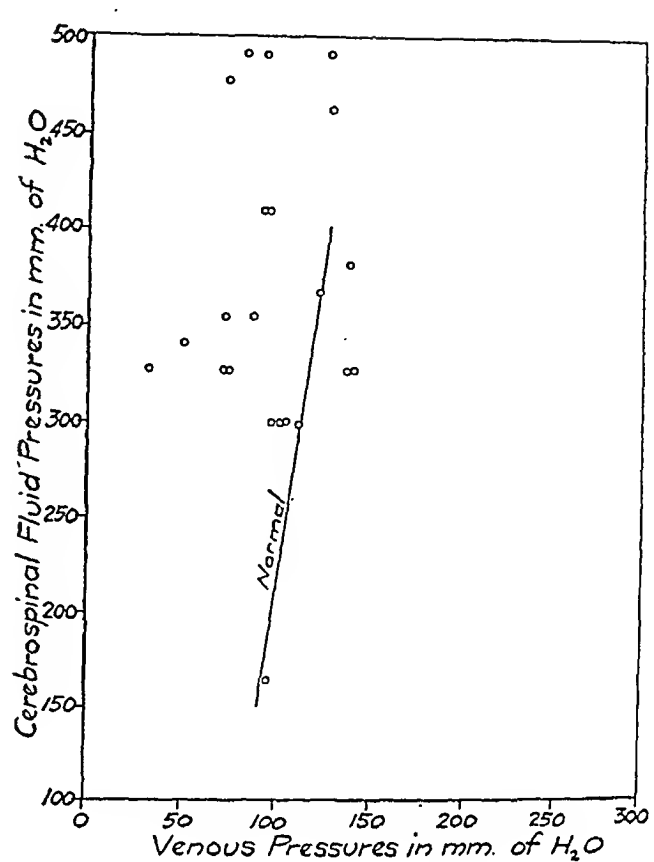


FIG. 3. CEREBROSPINAL FLUID PRESSURES AND VENOUS PRESSURES IN LEFT HEART FAILURE. (Before venesection.)

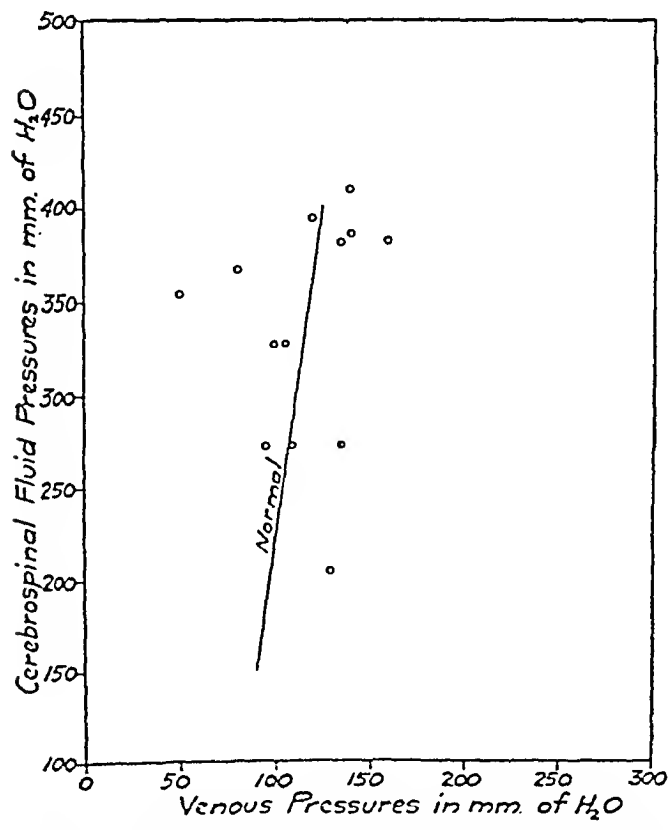


FIG. 2. CEREBROSPINAL FLUID PRESSURES AND VENOUS PRESSURES IN RIGHT HEART FAILURE. (After venesection.)

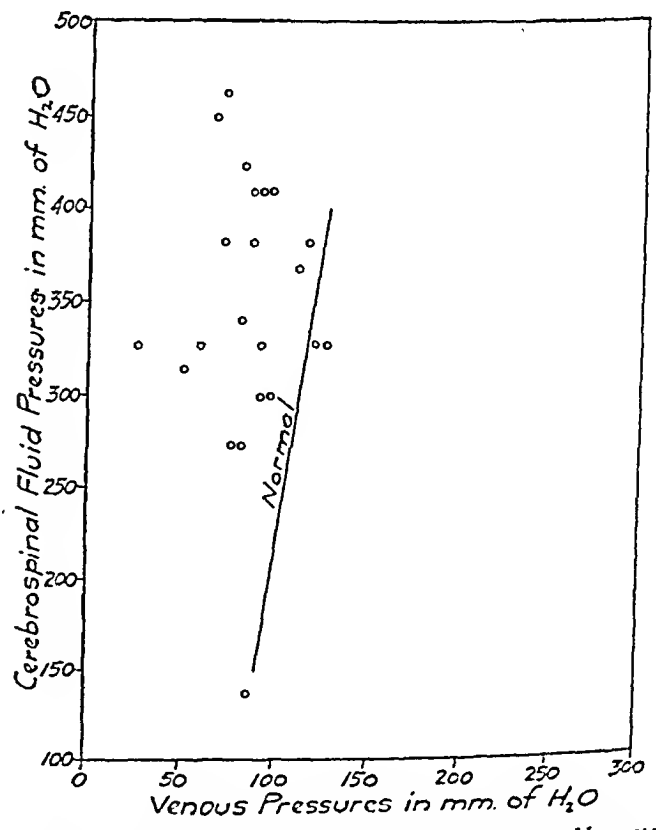


FIG. 4. CEREBROSPINAL FLUID PRESSURES AND VENOUS PRESSURES IN LEFT HEART FAILURE. (After venesection.)





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# MICROCOCCUS TETRAGENUS INFECTION

## I. REVIEW OF THE LITERATURE, REPORT OF A NON-FATAL CASE WITH SEPTICEMIA, MENINGITIS AND ARTHRITIS, AND BACTERIOLOGIC STUDIES

By HOBART A. REIMANN

(From Department of Medicine, University of Minnesota Hospital, Minneapolis)

(Received for publication November 28, 1934)

The appearance of only two or three reports (Greiwe, et al. (1), Steele (1)) of *Micrococcus tetragenus* infection in American medical literature would lead one to believe the disease to be rare and of little importance. It is probably quite common, but often disregarded or unrecognized in this country. More than 170 cases have been reported in Italian, French, German, English and Spanish journals.

*Micrococcus tetragenus*, after its discovery in 1881 by Koch and Gaffky, was believed to be a harmless saprophyte residing in the normal nasopharynx and often in tuberculous cavities in the lung. Its pathogenicity was first recorded by Jakowski (2) in 1886. Later Mya and Trambusti (3) reported two cases of septicemia, and Viquerat (4) produced suppuration by injecting cultures of *M. tetragenus* into blister sacs on tuberculous patients. The organism is, however, of comparatively low virulence and becomes invasive only under certain conditions in which the resistance of the host is reduced. When infection does occur, it resembles the local suppuration or septicemia with metastatic localization due to the closely related staphylococcus. The organism appears to have a predilection for serous membranes, but also invades parenchymatous organs.

All of the published reports, except two, deal with sporadic cases. Two small epidemics have been reported among soldiers during the World War; one by Birks, Thornley and Fawcus (5) who observed 25 cases of septicemia, and one by Trémolières and Loew (5) with 45 cases. In both series the mortality rate was low and the general features illustrated the importance of predisposing factors. In many of the cases, *M. tetragenus* was probably a secondary invader.

Clinically, the infection is characterized by features common to pyogenic infections due to

other cocci. The case reported here conforms to the prototype of the usual course. A brief summary of certain salient features of the disease, taken from reports giving adequate data in available journals, is given in Table I. Many French and Italian articles (6) are short and devoid of details.

The infection is apparently commonest in young adults. The onset is usually abrupt and is almost invariably preceded by some predisposing cause which presumably lowers resistance and makes invasion possible. Sore throat and respiratory tract infections are the commonest, as would be expected, since *M. tetragenus* often resides in the normal nasopharynx. Other predisposing factors are anemia (Mya and Trambusti (3), Arullani (7), Brugnola (1)), abscesses (Jakowski (2), Viquerat (4), Achard and Gailard (8), Steele (1) and others) and urinary tract infections (Gayet (9) and others). Interesting cases have been observed both preceding and following typhoid fever (Anglada (7), Laignel-Lavastine and Baufle and Meltzer (1)), several accompanying septicemia due to streptococci (Meltzer (1)) and one with brucellosis (10). The course of the infection may be mild or severe and is characterized by chills, sweats, remittent fever, moderate leukocytosis and often by splenomegaly. Fulminating cases have ended fatally on the seventh day (Leschke (1)), and protracted cases may last 7 months (Castaigne (1)). The mortality rate judged from 44 cases of septicemia in which the outcome is stated is 50 per cent.

Reports of more than 127 cases of septicemia are available, many of which also show localization in various parts of the body. Cases with septicemia alone are referred to in Table I and are reported in the papers of Mya and Trambusti (3), Arullani, Boni, Anglada, Tillaye, Marcora

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TABLE I  
Summary from literature of features of *Micrococcus tetragenus* infection

	Sex	Age	Predisposing factor	Onset	Duration	Outcome	Septicemia	Arthritis	Meningitis	Other localization	Other observations
<i>Cases with arthritis</i>											
Chauffard, A., and Ramond, F., Arch. de méd. Exper., 1896, 8, 304	F. M.	15 yrs. 18	Influenza	Gradual Sudden	11 days 10 days	Fatal Fatal	+	+		Endocarditis	Exanthem Bronchitis, empyema
Bertaux, A., cited by Lartigau (13)	M.	37	Sore throat			Recovered	+	+		Pneumonia, phlebitis	
Faisans and Le Damany, Semaine méd., 1897, 17, 258			Sore throat			Fatal		+		Pneumonia, empyema, exanthem	
von Ofenheim, E., Proc. Roy. Soc. Med., Clin. Sect., 1909, 2, 164	M.	38	Abscess	Gradual	5 months	Recovered	+	+		Exanthem	Typhoid fever and rheumatic fever first considered. No specific agglutinins; treated with vaccine
Stroebel, Beitr. z. klin. Chir., 1913, 83, 718	M.	26	Thoracotomy	Sudden		Recovered	+	+			
Leschke, E., Spez. Path. u. Therap. Kraus, Brugsch., 1919, 2, 1103	M.	23	Sore throat	Sudden	7 days	Fatal	+	+	+		
This report	M.	46	Sore throat	Sudden	7 weeks	Recovered	+	+	+	Prostate	Rheumatic fever and gonorrheal arthritis first considered. No agglutinins. Skin test negative
<i>Cases with pneumonia or empyema</i>											
Castalgne, J., Bull. Soc. Anat., 1897, 11, 394	F.		Fractured leg	Gradual	7 months	Fatal	+			Spleen and kidney abscess	
Bosc, F. J., and Galavielle, L., Arch. de méd. Exper., 1899, 11, 70	M.	45				Fatal					
Bosc, F. J., Arch. de méd. Exper., 1900, 12, 159	M.	21	Long illness, anemia	Sudden		Fatal	+			Peritonitis, enterocolitis	
Byers, J., and Houston, T., Lancet, 1913, 1, 1723	M.	11	A "cold"	Sudden			+			Exanthem	
<i>Cases with meningitis</i>											
Bezaneon, F., and Lepage, Semaine méd., 1898, 18, 40	F.	32	Following childbirth			Fatal	+		+		
Greiwe, J. W., Faekler, G. A., and Mitchell, E. W., Phila. Month. Med. J., 1899, 1, 528	M.	35		Sudden	3 weeks	Fatal			+		
Pende, N., Centralbl. f. Bakt., Ref., 1908, 41, 294	F.	48	Previously well	Sudden	5 weeks	Fatal			+		
Bonanno, A. M., Riforma med., 1931, 47, 363	M.	28		Sudden	4 weeks	Recovered	None		+		Believed at first to be brucellosis

TABLE I (continued)

	Sex	Age	Predisposing factor	Onset	Duration	Outcome	Septicemia	Arthritis	Meningitis	Other localization	Other observations
<i>Cases with septicemia with miscellaneous localization</i>											
Luigi, Fornaca, Riforma med., 1903, 19, 309 and 346	M.	37½ 45		Sudden	6 months	Recovered	+			Empyema	
Brugnola, A., Riforma med., 1906, 22, 957	F. F.	40 21	Anemia Anemia	Gradual Gradual	6 weeks —	Recovered Recovered	++ +				Patient not ill, afebrile
Debove, Presse méd., 1907, 15, 41	F.	33	Old endocarditis	Gradual	Months	Fatal	+			Acute endocarditis	
Ziesler, K., München med. Wchnschr., 1908, 55, 2487	F.	17	Sore throat	Sudden		Recovered	+				
Laignel-Lavastine and Bauffe, P., Compt. rend. Soc. de biol., 1909, 67, 661	F.	26	Typhoid fever	Sudden	1 week	Recovered	+				Occurred 12 days after typhoid fever
Caldera, C., and Pinaroli, G., Arch. ital. diotol., 1911, 22, 34	M.	16	Chronic mastoiditis	Sudden		Fatal	+			Empyema, lung gangrene	
Steele, A. E., J.A.M.A., 1914, 62, 930	M.	24			14 weeks	Fatal	+			Tibial abscess	
Lüdke, H., München med. Wchnschr., 1920, 67, 454	M. M. M.	21 34 41	Sore throat Typhoid fever Sore throat	Gradual Sudden	17 days	Recovered Recovered Recovered	++ ++ +			Petechia	Given specific vaccine
Kramár, E., Arch. f. Kinderh., 1930, 92, 248		3½ mos.			3 months	Recovered	+				Specific agglutinins in serum, 1-640
Adel, F., Med. Klin., 1931, 27, 129	M.	31	A "cold"	Gradual	2 months	Fatal	+			Petechia, endocarditis	First believed to be tuberculosis
Battistini, G., Riforma med., 1931, 47, 646	F.	24	Sore throat	Gradual	5 months	Recovered	+	Arthralgia			First believed to be rheumatic fever. Treated with vaccine
Meltzer, O., München med. Wchnschr., 1910, 57, 743	M. F. F.	35 27 24	 Postpartum Postpartum	Gradual Sudden Sudden	5 weeks 9 days 4 months	Recovered Fatal ?	++ ++ +				Specific agglutinins in serum 1-500 Streptococci in blood Streptococci in blood
Looten, J., and Ouf, Ann. d. Gynec., 1909, 6, 134	F.	24	Difficult labor	Gradual	6 weeks	Recovered	+	Arthralgia			Puerperal infection

(7), Ceraulo-Vetrano (10), Trémolières, Birks (5), Gayet (9) and others. Cases with localization in various parts of the body other than those listed in Table I are mentioned by the following authors: *arthritis* by Oettinger, Roger and Trémolières (11); *meningitis* by Vincent (12), Oettinger (11), Ramond (16), Rosenthal, von Riemsdk and Blum (12); *respiratory tract lesions* including mouth infections, pharyngitis, otitis media, bronchitis, pneumonia and empyema by Apert, Lewy, Lartigau, Park, Karlinsky, Steinhaus, Kapper, Netter, Delearde (13), Trémolières (5) and Kimmerle (14); *endocarditis*

by Sterling (15), Gaucher, Perfetti (16), Lenhartz, Meyer (17) and *pericarditis* by Benoit (13); *puerperal infection* by Cathala and Guéniot (18), Bondy, Hüsey, Wegelius (7); *peritonitis* by Pane (3) and Müller (19); *perinephritic abscess* by Vease (16); and *pyosalpinx* by Wollgreen (7). Battistini (1) reported glomerulonephritis during septicemia and cultivated *M. tetragenus* from the patient's urine. Exanthemata have been noted by Chauffard and Ramond, Faisans and Le Damany, Adel, von Ofenheim, Byers and Houston, Lüdke and Stroebel (1). Gaucher (16) noted purpura.

Only a few immunologic studies on patients have been made. Specific agglutinins were found in the serum by Meltzer, Brugnola (1) Trémolières (5) and Kramár (1); von Ofenheim and Debove (1) were unable to demonstrate significant titers. Several authors report beneficial effects from vaccine therapy (Lüdke, Battistini (1), Gayet (9)), others were unsuccessful (von Ofenheim (1)). Vaccine therapy in experimental animals was ineffectual (Lambea (20)).

**Diagnosis.**—Since the infection follows other diseases, as it often does, it is difficult to recognize for there are no pathognostic features aside from those common to other pyogenic infections. It has been confused with rheumatic fever, gonorrheal arthritis, tuberculosis, typhoid fever, malaria and sepsis due to other pyogenic cocci, especially the staphylococcus. At present, etiologic diagnosis can only be made by the isolation and identification of *M. tetragenus* from the blood or from other foci of infection.

#### CASE REPORT

On June 14 and 15, G. J., a fisherman, aged 46, drank alcoholic liquor excessively and awoke on the morning of the sixteenth feeling ill with a severe sore throat, headache, and alternating chilliness and sweating. He went to work, but soon became worse and retired because of

knee was slightly swollen and painful. The overlying skin was neither reddened nor hot. The heart and lungs were normal. The prostate was tender but not enlarged and several drops of viscid white pus were expressed from the urethra on two occasions. No abnormal neurologic signs were noted. The clinical impression was acute rheumatic fever or gonorrheal arthritis.

**Course.** The arthralgia and fever were uninfluenced by amidopyrine. On June 27, the tenth day of illness, the right knee became larger and 40 cc. of mucinous, lemon yellow, turbid pus was aspirated. The right wrist was aspirated but no pus was obtained. The knee was aspirated again on the eighteenth and twenty-second days; 60 cc. and 9 cc. of pus were removed. The patient's condition improved somewhat until the fifteenth day when generalized muscular twitching, irrationality, somnolence and pupillary inequality appeared. His neck was held rigid, the deep reflexes were hyperactive, but Babinsky's sign was normal. Lumbar puncture revealed cloudy fluid under 18 mm. mercury pressure; 8 cc. were withdrawn. Spinal drainage was repeated at intervals 10 or 12 times thereafter. The patient voided involuntarily and the stools were diarrhoeal for a day.

Irrationality and somnolence persisted with brief, apparently lucid intervals. On the seventeenth day he complained of severe pain in the left shoulder. His neck was rigid and nystagmus was noted. He appeared to be moribund on the eighteenth day, but thereafter gradually improved. On the twenty-fourth day two shaking chills occurred. At times he was irrational, but the temperature gradually declined and remained normal after the

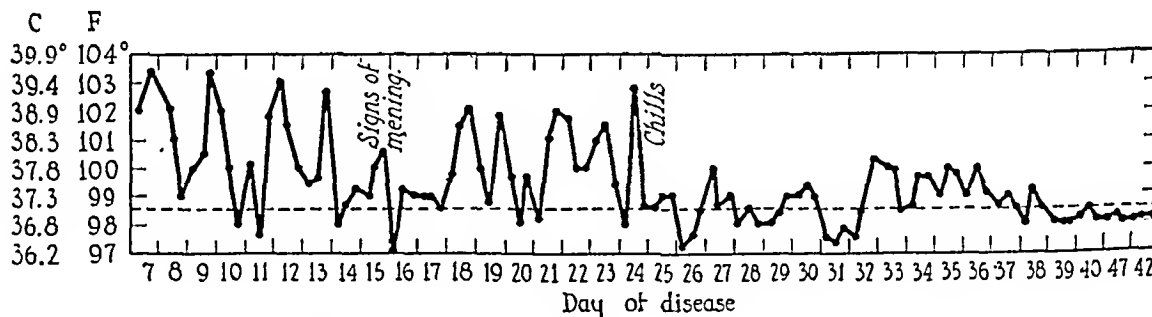


FIG. 1. TEMPERATURE CHART OF PATIENT WITH *M. tetragenus* SEPTICEMIA, ARTHRITIS AND MENINGITIS.

backache and pain in his limbs. Headache was severe and constant. There was transient earache and partial deafness. After a day or two, pain localized in the right knee, shoulder and arm. On several occasions thereafter the backs of both hands became temporarily swollen and painful. His condition remained unchanged until he entered the hospital on the seventh day of illness. His past history was irrelevant except for gonorrhea in 1931.

Physical examination revealed a moderately ill, febrile patient evidently suffering from pain in his knee and shoulder. The pharyngitis had disappeared. There was tenderness and swelling of the right wrist, upper arm and shoulder, tenderness in the left shoulder, and the right

thirty-eighth day. The fever was remittent and irregular as shown in Figure 1. The pulse rate varied between 70 and 110.

**Laboratory observations.** There was slight albuminuria. The leukocytes ranged between 12,000 and 18,000 per cu. mm. The red cell count and hemoglobin were unchanged. The blood Wassermann was negative. The spinal fluid in the early period contained between 1400 and 3400 cells, mostly polymorphonuclear neutrophils, between 8 and 40 mgm. sugar, and the Pandy and Nonne tests were positive. The cell count gradually diminished to 21 on the sixty-fourth day when the last fluid was withdrawn at which time 55 mgm. of sugar were present.

## BACTERIOLOGIC STUDIES

**Blood cultures.** A blood culture in broth made on the ninth day showed gram-positive, irregularly shaped cocci in small clusters and a few in diploform after 24 hours at 37° C. When subcultured on blood agar, a few minute colonies of small gram-positive cocci which were thought to be staphylococci grew in 24 hours. They were believed to be secondary invaders or contaminants and were not studied further. Subsequent blood cultures in broth made on the sixteenth and nineteenth days were sterile, but on one agar pour-plate two whitish colonies appeared after 3 days. The colonies after a week at 37° C. became yellow. The cocci composing each of these colonies were of two distinct kinds, large and small, and both occurred in tetrad formation (Figure 2A).



FIG. 2(A). LARGE AND SMALL TETRADS COMPOSING A YELLOW COLONY OBTAINED FROM A BLOOD CULTURE ON THE SIXTEENTH DAY OF ILLNESS. (B) CLUSTERS OF PLEOMORPHIC COCCI SURROUNDED BY A HALO (CAPSULE?) IN PUS FROM THE KNEE ASPIRATED ON THE TENTH DAY. (C) SMALL DIPLOCOCCI AND TETRADS (CAPSULATED?) OBTAINED FROM BROTH CULTURE OF SPINAL FLUID ON THE FIFTEENTH DAY OF ILLNESS.

All magnified  $\times 1150$ .

**Pus from the knee.** In a smear made from pus aspirated on the tenth day, 3 clusters of gram-positive, pleomorphic cocci were found after prolonged search (Figure 2B). One or two of the larger cocci were divided by a transverse fissure. The clusters appeared to be enclosed in a faintly staining pink capsule-like material. On a blood-agar plate inoculated with pus, one small whitish colony appeared composed of gram-positive cocci mostly in tetrad formation. Similar colonies appeared on plates streaked with pus removed on the eighteenth day. Cultures made from pus aspirated later were sterile.

**Spinal fluid.** Spinal fluid obtained on the fifteenth day was inoculated into 3 plain-broth tubes

and on blood-agar plates. After 5 days incubation a slight stringy growth appeared at the bottom of 2 tubes. Smears from these cultures showed a few partly decolorized and some entirely gram-negative cocci, irregular in shape and size, mostly in tetrad formation. On subculture from one tube on a blood-agar plate, 14 small white colonies appeared after 18 hours, composed of gram-positive small tetrads. A halo surrounded the tetrads (Figure 2C) which suggested a capsule, but none was stained nor demonstrable with India ink. Organisms from this colony were subcultured on blood-agar plates. After 48 hours, large greenish colonies surrounded by a green zone appeared. On several other blood-agar plates, hemolysis was noted where the colonies were crowded. On subculture on blood-agar or in blood-broth tubes hemolysis failed to recur. The colonies were composed of typical gram-positive tetrads. Similar results were obtained from cultures of spinal fluid obtained on the eighteenth day. At this time, one large white colony appeared on a plain-agar plate after 48 hours at 37° C. After centrifugation of a sample removed on the twenty-seventh day, a few diplococci were seen for the first time in a direct smear. Thereafter, similar organisms were cultivated from each of the subsequent samples of spinal fluid by Dr. R. Koucky. The last culture was obtained on August 20, three weeks after the patient had become clinically well.

**Urethral pus.** Pus expressed from the urethra on two occasions showed a great predominance of gram-positive cocci, arranged singly, in pairs, in threes and many in tetrads and small clusters. Cultures on plain-agar after 24 hours at 37° C. formed a heavy growth chiefly of whitish colonies composed of large gram-positive tetrads.

Rabbits, guinea pigs and white mice inoculated intraperitoneally with whole blood, spinal fluid, pus from the knee and with subcultures of the organism isolated from the three sources all survived. No agglutinins for the organism or any of its variant forms appeared in the patient's blood obtained at 5 intervals during and after his illness. A dilute suspension of cocci killed by heat at 60° C. for  $\frac{1}{2}$  hour and injected intracutaneously in the patient failed to cause erythema. The general characteristics of the iso-



TABLE II

*Cultural characteristics of M. tetragenus according to several authorities compared with the strain isolated from the case reported*

Author	Form	Colonies on agar	Growth in broth	Gelatin liquefied	Acid in					Nitrate reduced	Hemolysis	Pathogenic for mice
					Dextrose	Sucrose	Maltose	Lactose	Milk			
Sterling, S., <i>Centralbl. f. Bakt.</i> , 1896, 19, 141	Capsulated tetrads in blood culture	Yellow, round		+					0			
Leschke, E. (1)	Tetrads, capsule present in vivo and on solid media	Greyish-white, slimy. Zone of hemolysis on blood-agar		0							+	+
Bergey, D. H., <i>Manual of Determinative Bacteriology</i> , Williams and Wilkins Co., Baltimore, 1925, 2nd ed.	Tetrads in vivo, pairs or irregular masses in media, pseudocapsule	White, smooth, glistening	Clear, gray sediment	0	A			A	A±	0		+
<i>System of Bacteriology</i> , Med. Res. Council, London, 1929, 2, 26	Large capsulated tetrads in vivo; smaller and single or pairs or groups on media	Surrounded by green area on blood-agar	Clouding first then a deposit	0	Variable							+
<i>Principles of Bacteriology and Immunology</i> , Topley, W. W. C., and Wilson, G. S., Arnold, London, 1929, 1, 393	Capsulated tetrads	Glutinous, difficult to emulsify	Thick, weedy glutinous deposit	0	A	A	A	A				+
<i>Textbook of Bacteriology</i> , Zinsser, H., and Bayne-Jones, S., Appleton-Century Co., New York, 1934, 7th ed., p. 310	Capsulated tetrads	Transparent at first, later grayish-white	Evenly clouded	0					A			+
This report	White colonies—large cocci in tetrads or in groups. No capsule	White, occasionally with greenish tinge and halo on blood-agar	Granular sediment. Spirals on shaking	0	A	A	A	A	0	+	0	0
	Yellow colonies—large cocci, usually all in tetrads. No capsule	Yellow large	Granular yellow sediment	+ in 3 wks.	0	0	0	0	0	0	0	0
	Translucent colonies—cocci pleomorphic, not in tetrads. No capsule	Smaller, yellow-blue iridescence	Diffuse with sediment. Spirals on shaking	+ in 2 days	A	A	A	0	0	0	+	0

lated micrococcus are summarized in the lower portion of Table II.

#### *Variation of the isolated organism*

The striking variations observed in the morphologic characteristics of the cocci and of the colonies at once suggested the operation of dissociative phenomena. On one blood-culture agar-plate the colonies were composed of large and small cocci (Figure 2A). When cocci from this colony were spread on another agar-plate, two types of colony appeared, large porcelain-like cream-colored colonies and a sparse growth of small white colonies (Figure 3B). An agar-plate seeded from a large white colony was permitted to age at room temperature. After 48 hours many of the isolated colonies became sulfur yellow while those crowded together remained smaller and white. After 5 days, opaque yellow "daughter" colonies appeared on some of the large yellow

forms. On the twelfth day the colonies were all a golden yellow, and composed of large tetrads. A whitish halo appeared around some of the smaller forms. On the halo, white "daughter" colonies grew and in the yellow center, yellow "daughter" colonies (Figure 3A) developed.

When organisms from the small colony were seeded on agar, small opaque white colonies slowly appeared. The cocci were single, in diploform or in small clusters, pleomorphic and metachromatic (Figure 3E). When the plate was examined with a 10× hand lens after 72 hours, numerous minute transparent colonies less than 0.1 mm. in diameter became visible scattered among the larger (1.5 mm.) whitish ones. Cocci from these colonies were markedly pleomorphic and many were completely decolorized by Gram's method. The cocci varied in size from those as large as the one illustrated in Figure 3D to ones just within the range of vision of the oil immersion system (Fig-

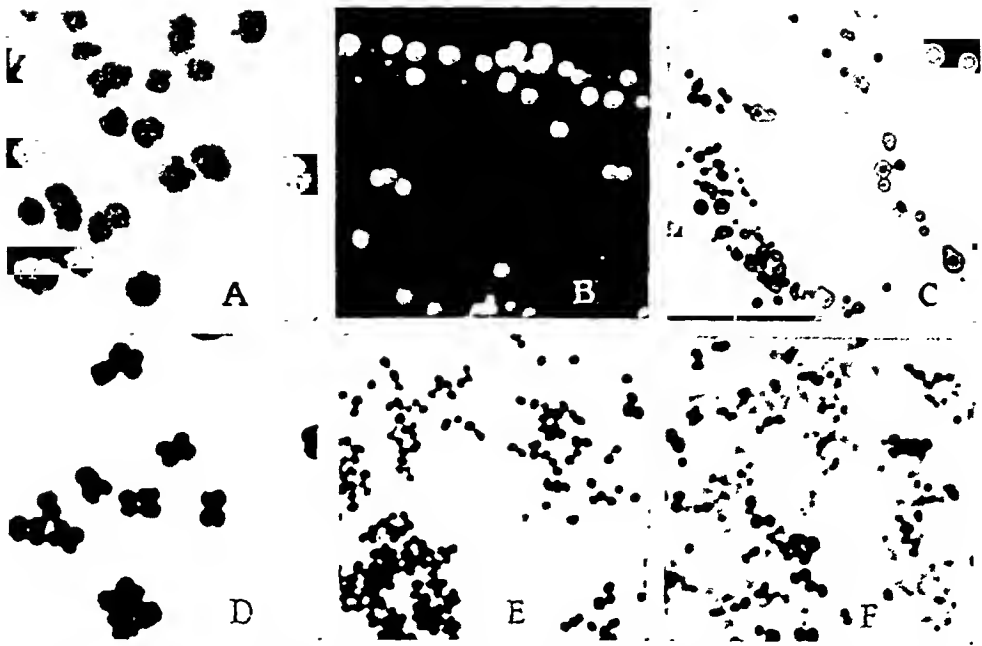


FIG. 3(A). COLONIES 2 WEEKS OLD WHICH ORIGINALLY WERE WHITE LIKE THOSE OF B. After aging they became yellow and later developed a whitish halo-like outgrowth. On the yellow center are yellow "daughter" colonies, on the halo are white "daughter" colonies. (B) LARGE AND SMALL WHITE COLONIES. (C) LARGE AND SMALL TRANSLUCENT COLONIES, (All slightly magnified.) (D) LARGE COCCI IN TETRADES COMPOSING THE YELLOW COLONIES. (E) SMALL COCCI SINGLE AND IN CLUSTERS COMPOSING YOUNG WHITE COLONIES. (F) PLEOMORPHIC, METACHROMATIC COCCI COMPOSING THE TRANSLUCENT COLONIES.

ure 3F). After 5 days incubation, the minute colonies increased to 1 mm. in size and became translucent with a yellow-blue iridescence. Subculture produced colonies which grew more rapidly and were translucent. At various times during subsequent subcultures an occasional large white colony or a large yellow one would appear among the myriads of translucent colonies.

Thus from the blood culture at least three distinct, fairly stable forms of colony were obtained, (1) white, (2) yellow and (3) translucent. Other less stable varieties were observed. By special methods to be reported elsewhere, the translucent form was induced to dissociate into a shell-pink variety and an orange-buff variety, each of which bred true on repeated subculture. There was evidence of constant reversion of one form into another. Similar results were observed with the organism isolated from the spinal fluid and knee pus, and with several strains of *M. tetragenus* obtained from culture collections in Vienna and Chicago.

The biological reactions of the organisms com-

posing the three varieties of colony varied considerably as shown in Table II. Cocci from the white colonies were large, usually occurred in clusters or tetrads, produced greenish color on blood-agar, did not liquefy gelatin, but fermented dextrose, sucrose, maltose and lactose, and reduced potassium nitrate. Cocci from the yellow colonies were in large and small tetrad formation, were chromogenic, liquefied gelatin slightly after 3 weeks, did not ferment any sugar, or reduce nitrate. Cocci from the translucent colonies were markedly pleomorphic and rarely occurred in tetrads; they liquefied gelatin in 2 days, fermented dextrose, sucrose and maltose but not lactose, did not reduce nitrate and were hemolytic. None of the strains fermented mannite, acidified milk nor formed indol. None were pathogenic for mice. Capsules could never be demonstrated with certainty.

#### *Classification of the organism*

Criteria for the identification of *M. tetragenus* vary considerably according to different authori-

ties as a glance at Table II will reveal. No two agree in all respects. In general, the organism is said to occur in large gram-positive, capsulated tetrads *in vivo* and to become smaller and lose both capsule and tendency to group in fours on subculture *in vitro*; the colonies are glistening-white; gelatin is not liquefied, milk is not coagulated, blood cells are not hemolyzed, and no gas is produced in various sugar media. The points of variance are the production of yellow and red pigment (Chauffard and Ramond (1), Sterling (2), Boschi and Bellei (13), Laignel-Lavastine and Baufle (1), Roger and Trémolières (5), Brugnola (1, 8, 20, 21, 22); lack of virulence for animals (Teissier, Boutron (22), Debove, Brugnola, Ziegler, Kramár (1)); liquefaction of gelatin (Sterling (15), Jacobelli (22), Chauffard and Ramond, Laignel-Lavastine and Baufle (1)); coagulation of milk (Bergey, Zinsser and Bayne-Jones (15)); hemolysis (Leschke (1)) and capsule formation (Bergey (15)).

Practically all of these apparent contradictions can be explained on the basis of microbic dissociation. Much confusion could have been avoided had the early studies of Boschi and Bellei (13) and Jacobelli (22) been more seriously considered. These observers anticipated the modern conception of bacterial variation nearly 40 years ago when they showed that colonies of *M. tetragenus*, aged on agar plates, formed yellow pigment and that after subsequent transfer, while colonies again appeared. Teissier (22) in 1896 even noted the formation of "daughter" colonies. For a time it was controversial as to whether many different strains existed or if the different forms were all variants of a single strain. It seems now that both views were correct; numerous strains exist and each may dissociate into various forms.

There has been considerable difficulty in differentiating *M. tetragenus* from the staphylococcus to which it is closely related. The differences between typical undissociated strains of either type are distinct. The dissociated forms of one may, however, resemble either the undissociated form of the other or its variants so closely that identification is almost impossible, except if reversions to the original form occur. For example, Hoffstadt and Youmans (23) have dissociated the *Staphylococcus aureus* into a number of variant forms,

some of which were no longer chromogenic, no longer liquefied gelatin and no longer caused hemolysis. These are reactions also characteristic of *M. tetragenus*. On the other hand, as I have shown, one of the dissociated forms of *M. tetragenus* liquefied gelatin, caused hemolysis and its cocci grew in clusters and produced colonies with a blue-yellow iridescence; it was thus indistinguishable from the staphylococcus except that milk was not acidified and that occasional reversions to the original form occurred.

#### COMMENT

Increased knowledge of microbic dissociation has explained many of the older difficulties in the classification of bacteria. When comparing one species of organisms with another it is necessary to deal with known variants of each. As shown in this study, the "typical" undissociated forms of *M. tetragenus* and *S. aureus* are distinct, whereas the dissociated forms of one may be indistinguishable from those of the other. Similar phenomena have been encountered in the study of other bacteria. The relation of microbic dissociation to infection, disease and recovery is but little understood. It is of interest to speculate as to the form or phase or cyclostage in which *M. tetragenus* existed in the patient described in view of the fact that the organism was seen in its "atypical" (?) form in the pus from the knee and spinal fluid and because of its slow growth on first subculture and its presence in the spinal fluid after the patient had apparently recovered. Further studies in this regard and a detailed description of the five variant forms will be presented elsewhere.

The isolation of a bacterium which grew in colonies of different character each composed of cocci of different size, shape, arrangement and biologic reactions from the same patient would lead one to suspect a mixed infection or contamination. It became evident that a single species with its variant forms was concerned after the transformation of one form of colony into another on culture media had been watched. It is possible that cases of *M. tetragenus* infection are occasionally missed because of the variability of the isolated organism, or without further study cases are easily mistaken for staphylococcus infection.

## SUMMARY

A nonfatal case of *M. tetragenus* infection with septicemia, purulent arthritis and meningitis is described. The organisms isolated from the patient's blood, pus from the knee and from the spinal fluid conformed in general to the criteria established for the species *Gaffkyia tetragena*. From the typical white colonies isolated, two variant forms developed, one yellow, the other translucent; each form of colony was composed of cocci of different but constant characteristics. Reversion or transformation of one form into another on culture media was observed. The biologic characteristics of the variant forms vary considerably, which helps to explain the apparently contradictory criteria of numerous authorities regarding the differentiation of *M. tetragenus* from staphylococci.

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# THE FATE OF PEOPLE WITH UNEXPLAINED GASTRIC ANACIDITY

## FOLLOW-UP STUDIES

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Until recent years it was generally believed that gastric anacidity indicated disease of clinical importance even though the patient at the moment presented no serious symptoms (1). Recently, however, as a result of extensive studies on more or less normal people it has become apparent that failure of the stomach to secrete acid, or for that matter any juice at all, even after so powerful a stimulus as histamine, is compatible with good health. Under the caption of "unexplained anacidity" we described several years ago a group of patients without gastrointestinal symptoms and without cancer of the stomach or pernicious anemia in whom anacidity after histamine was demonstrated as an accidental finding during the course of routine hospital studies (2). It seemed of importance to follow the subsequent course of these people with regard to their general state of health, and particularly to see whether they developed cancer of the stomach, pernicious anemia, or hypochromic anemia, conditions of which gastric anacidity has been claimed to be a precursor (3).

### MATERIAL

The subjects were of the same type as those described in the previous paper (2), the general run of hospital patients. We excluded at the start (a) those in whom there was any suspicion of pernicious anemia, (b) those who had a hypochromic anemia with hemoglobin below 80 (Sahli), (c) those with gastro-intestinal symptoms more outspoken than the minor complaints of gas or occasional epigastric fullness, so common in any group of clinic patients, and (d) those with cancer of the stomach.

The presence of anacidity was established by the histamine test (4), and gastro-intestinal roentgenography was carefully done, aside from the usual general examination. Forty-three cases were followed for periods of from one to seven years in the anacidity clinic. Physical examinations, blood studies, and gastro-intestinal

x-rays were made from time to time and the histamine test was repeated in many instances. Only one patient was lost track of during the period of study. Another died of a stroke. There were twenty-two females and twenty-one males. Only six of the whole group were under 40 years of age; most of them were in the period when pernicious anemia and cancer are common.

### *Development of cancer of the stomach in people with gastric anacidity*

The duration of the follow-up with reference to development of cancer of the stomach was as follows:

Years followed .....	1	2	3	4	5	6	7	8	9	10	11	12
Number of patients ...	3	4	12	16	2	4	1	1*				1†

\* Anacidity to Ewald Meal in 1924; first histamine test in 1929.

† Anacidity to Ewald Meal in 1922; first histamine test in 1930.

*In no case did cancer of the stomach develop.* In most instances gastro-intestinal x-rays were repeated toward the end of the follow-up period, in nearly all, the patient was seen and examined, in a few, a report which seemed conclusive came by letter.

### *Development of pernicious anemia in people with gastric anacidity*

The duration of the follow-up with reference to development of pernicious anemia was as follows:

Years followed .....	1	2	3	4	5	6	7	8	9	10	11	12
Number of patients .....	3	3	12	15	2	4	1		1			1

In nearly every case the patient was carefully examined at the end of the follow-up period for physical evidences of pernicious anemia (tongue, spleen, cord changes) and blood studies were made. In a few instances the patient could not return but wrote about his condition in reply to a questionnaire. *In none of the group was there*

any suggestion that pernicious anemia had developed.

During the course of the study, however, there came to our attention two patients who deserve a word of comment. Neither is included in the series reported above.

*E. S.* (No. 194344), a salesman, 48 years old, came to the clinic in November 1929 for mild epileptiform attacks. There were no symptoms or physical signs to raise any suspicion of pernicious anemia. There were no gastro-intestinal symptoms but the histamine test showed an anacidity with practically no gastric secretion. Blood: (November 1929) R.B.C. 5.4 M, Hb. 17.2 grams per cent, W.B.C. 9200, no morphological abnormalities. April 1931. Patient perfectly well. Blood: R.B.C. 4.98 M, Hb. 16.6 grams per cent, C.I. 0.99, W.B.C. 9200. Platelets 313,000. Price-Jones curve normal. June 1932. Patient well. Second histamine test, complete anacidity. Blood: R.B.C. 5.2 M, Hb. 17.5 grams per cent, C.I. 0.97, W.B.C. 9400. Platelets 387,000. There was a definite tendency to macrocytosis. The Price-Jones curve was shifted to the right and there were a good many cells with diameters between 9 and 10  $\mu$ .

The question arose as to whether one was dealing with the earliest evidences of pernicious anemia, but in 1934 the patient who had had no liver therapy was perfectly well.

The second case was that of *J. A.*, a man of 45 years who came to the clinic in January 1930 with the complaint of diarrhea off and on for six years. Physical examination was not remarkable aside from acne rosacea and slight undernutrition. Gastro-intestinal x-ray studies were normal. Barium enema, normal. Histamine test showed complete anacidity. Blood: R.B.C. 4.7 M, Hb. 14.3 grams per cent, W.B.C. 8,900. No morphological changes. The diarrhea stopped after administration of dilute hydrochloric acid; the man gained twenty-five pounds and has remained well. April 1931. Blood: R.B.C. 4.6 M, Hb. 15.5 grams per cent, C.I. 0.98, W.B.C. 8800. Morphology normal. Price-Jones curve, normal. April 1932. Blood: R.B.C. 4.6 M, Hb. 15.7 grams per cent, C.I. 0.99, W.B.C. 8600. There was marked anisocytosis with many macrocytes, the Price-Jones curve being markedly shifted to the right. August 1932. Blood: R.B.C. 4.7 M, Hb. 16.9 grams per cent, C.I. 1.04, W.B.C. 7800. The cells showed marked anisocytosis and slight polychromasia. The average red cell diameter was 8.07  $\mu$ . There were many large cells.

Here again the suspicion of early pernicious anemia was aroused, but since the patient is perfectly well two years later no conclusion can be reached.

#### *The development of hypochromic anemia in patients with anacidity*

During the course of this study we encountered six cases of hypochromic anemia with anacidity.

They presented the usual features of the syndrome with prompt improvement of the blood on iron therapy, and relapse when iron was discontinued for any length of time. These patients were excluded at the start from the group of "unexplained anacidities" with which we are dealing in the present report. Of the patients with anacidity who were not anemic at the start of the follow-up period none developed hypochromic anemia. One patient on one occasion had only 11.2 grams of hemoglobin per cent but subsequently his blood was normal without administration of iron. This fact, that none of the patients became anemic, seems of considerable interest since it suggests that some influence other than anacidity is of major importance in precipitating the blood changes. We have discussed this matter in detail elsewhere (5).

#### *Permanence of anacidity*

In our experience anacidity following an injection of histamine is almost always permanent; restoration of anything approaching a normal gastric secretion is out of the question (6). In the present series the histamine test was repeated one or more times after varying intervals (one to four years) in 25 cases. Anacidity persisted in all but one. This was a man of 60 years who secreted no acid in June 1931 but in March 1934 he attained a free acid of 31 and total acid of 44. Whether there was an actual restoration of slight secretory ability or whether a small amount of acid was obscured by mucus on the first test can not be said definitely.

#### *General course of events in patients with unexplained anacidity*

As we followed these patients year after year nothing of note developed in regard to their general condition; certainly one did not get the impression that anacidity led to the development of any clinical symptoms, or that it shortened life. Patient *Ed*, for example, who was 67 years old at the start of the study has been followed for 4 years with no deterioration of health. Patient *Ko*, first seen six years ago, had a cholecystectomy and has been well ever since. Patient *El*, 75 years old at the start of the follow-up period, with arteriosclerosis and myocarditis, remains about the same four years later.

## DISCUSSION

Forty-three clinic patients, for the most part with minor disabilities, in whom gastric anacidity was discovered as an accidental finding were carefully followed over periods varying up to seven years. In no case was there evidence that the absence of gastric secretion led to the development of any particular symptoms or to general impairment of health; in no case did cancer, hypochromic anemia or pernicious anemia supervene. The anacidity persisted except in one instance in which after an interval small amounts of acid were demonstrated.

While these observations in no way contradict the general view that gastric anacidity may be a precursor of cancer of the stomach or of pernicious anemia, they indicate that the hazard to the individual is very small. Statistics also make this clear. Thus the death rate from pernicious anemia from 1921 to 1929 is reported as about 8.6 per 100,000 (7), and if one assumes that before the introduction of liver therapy the average duration of life was five years, then one might conclude that the incidence in the population at large was 43 per 100,000. But among 100,000 people in the pernicious anemia age period there are at least 15,000 with anacidity (8). Hence the chance on a statistical basis alone of any individual with anacidity developing pernicious anemia is only about 3 per 1000, or roughly one in three or four hundred.

The fact that none of these individuals with deficient gastric secretion developed pernicious anemia requires a further word of comment in view of the current theories of pathogenesis of this disease. The view seems generally accepted in this country, following the work of Castle, that the anemia results from the absence of a substance normally formed by interaction of a constituent of the gastric juice (intrinsic factor) with ingested protein (beef). The intrinsic factor according to Castle is independent both of acid and of pepsin and the question comes up whether it is present in the stomachs of these patients with anacidity who do not develop pernicious anemia. The data on this point are in conflict. Castle reported two such patients in whose gastric juice he was able to demonstrate the intrinsic factor in spite of anacidity (9). Barnett in our clinic, on the other

hand, was unable to secure it from the secretions of several pedigreed cases of simple anacidity without anemia (10). A priori, it seems improbable, if not impossible, that most of the patients with unexplained anacidity can secrete the "intrinsic factor"—at least in quantity enough to serve any practical purpose. It should be recalled that most of these people not only secrete no acid but really produce no gastric juice at all (11). Even after histamine there is no response, and aspiration over a period of an hour yields only a few cubic centimeters of mucoid material. Extraction of gastric contents after a meal of beef shows that the food is not digested and even if traces of "intrinsic factor" should be present it is difficult to see how they could mechanically come in contact with the ingesta in such a way as to liberate the anti-anemic substance. Be this as it may, the two patients with unexplained anacidity in whom Barnett was unable to demonstrate the "intrinsic factor" in 1931 have been followed up to the present time without showing any signs of pernicious anemia. The crucial experiment of course would be an assay for anti-anemic substance of the livers of achylia patients without anemia, and this we hope to do when material is available.

All this to our minds indicates that the absence of Castle's intrinsic factor in the gastric juice does not necessarily lead to the development of pernicious anemia. Apparently only an occasional person needs the protection of the Castle-Minot anti-anemic substance to safeguard himself against the disease whatever the ultimate cause of it may turn out to be. This view is supported by reports of blood changes after gastrectomy resembling those of pernicious anemia (12). The rare cases in which this occurs only serve to emphasize the vastly greater number of gastrectomies following which pernicious anemia does not develop. So too, very occasionally, when the gastric mucosa is seriously altered in connection with cancer of the stomach, does pernicious anemia develop (13), but in the vast majority of cases anemia is not of the Addisonian type.

## CONCLUSIONS

Forty-five patients with unexplained anacidity as previously defined were followed over periods of from one to seven years. In no case did



cancer of the stomach, hypochromic anemia, or pernicious anemia supervene. The implications of these findings especially with reference to the etiology of pernicious anemia are discussed.

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# THE RELATION OF UPPER RESPIRATORY INFECTIONS TO RHEUMATIC FEVER IN CHILDREN

## I. THE SIGNIFICANCE OF HEMOLYTIC STREPTOCOCCI IN THE PHARYNGEAL FLORA DURING RESPIRATORY INFECTION<sup>1</sup>

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The view that the rheumatic process is initiated or activated by streptococcal respiratory infections has met with wide acceptance. The evidence which has accumulated in support of this conception originated in the long recognized association of tonsillitis and rheumatic fever, as well as the so-called rheumatic sequelae of scarlet fever.

The more recent observations of Glover (1), Schlesinger (2), Coburn (3), Collis (4) and others have stressed a *broadier view of streptococcal respiratory infections* and have included such infections as pharyngitis, common cold, sinusitis, otitis, bronchitis and cervical adenitis. It is important to note that the presence of hemolytic streptococci in the flora of the throat during these infections has been considered by many observers as diagnostic of a streptococcal respiratory infection, the criteria for diagnosis being bacteriologic rather than clinical. Coburn and Pauli (5) from extensive epidemiological, bacteriological and immunological studies postulate that the hemolytic streptococcus initiates the rheumatic process.

The majority of investigators emphasize the *quiescent period* (of apparent health), a period of approximately seven to twenty-one days between the respiratory infection and the subsequent rheumatic recurrence, lending support to the hypothesis that rheumatic fever is allergic in nature. Paul and Salinger (6) from historical family studies have described simultaneous waves of respiratory infections and rheumatic recrudescences in several members of the household. Similar observations have been reported by various investigators, occurring in schools, camps, hospital wards and convalescent homes. In some instances

these episodes have assumed epidemic proportions.

It must be conceded that these various observations appear quite convincing, but their possible specific etiological significance may be questioned in view of the known frequency of respiratory infections in children in the seasons when rheumatic activity is likely to occur. This is illustrated by the following observations conducted for a two year period, 1930 to 1932, in which 222 ambulatory rheumatic subjects five to fifteen years of age were under close observation as part of another investigation. These children were under the medical supervision of the Heart Clinic of the New York Nursery and Child's Hospital and were seen at least monthly. The majority of the subjects were under our care from the onset of the first rheumatic symptom. Every effort was made to record accurately any illness suffered by these children.

The seasonal incidence of respiratory infections and rheumatic recurrences experienced by these subjects is graphically presented in Chart 1.

Here may be noted the three seasons of increased incidence of respiratory infections—fall, winter and spring—common in this section of the country. The trend of the curves representing the seasonal incidence of rheumatic recurrences, while somewhat similar to the respiratory curve, is significantly lower, showing the greatest incidence in the spring season. During this two year period 222 subjects experienced 783 respiratory infections and 401 rheumatic recurrences. It is of some significance that less than 10 per cent of these rheumatic episodes were preceded within a period of three weeks by a respiratory infection. Bacteriological studies of the pharyngeal flora were not made in those years.

<sup>1</sup> This study was conducted under a special grant from the Commonwealth Fund.

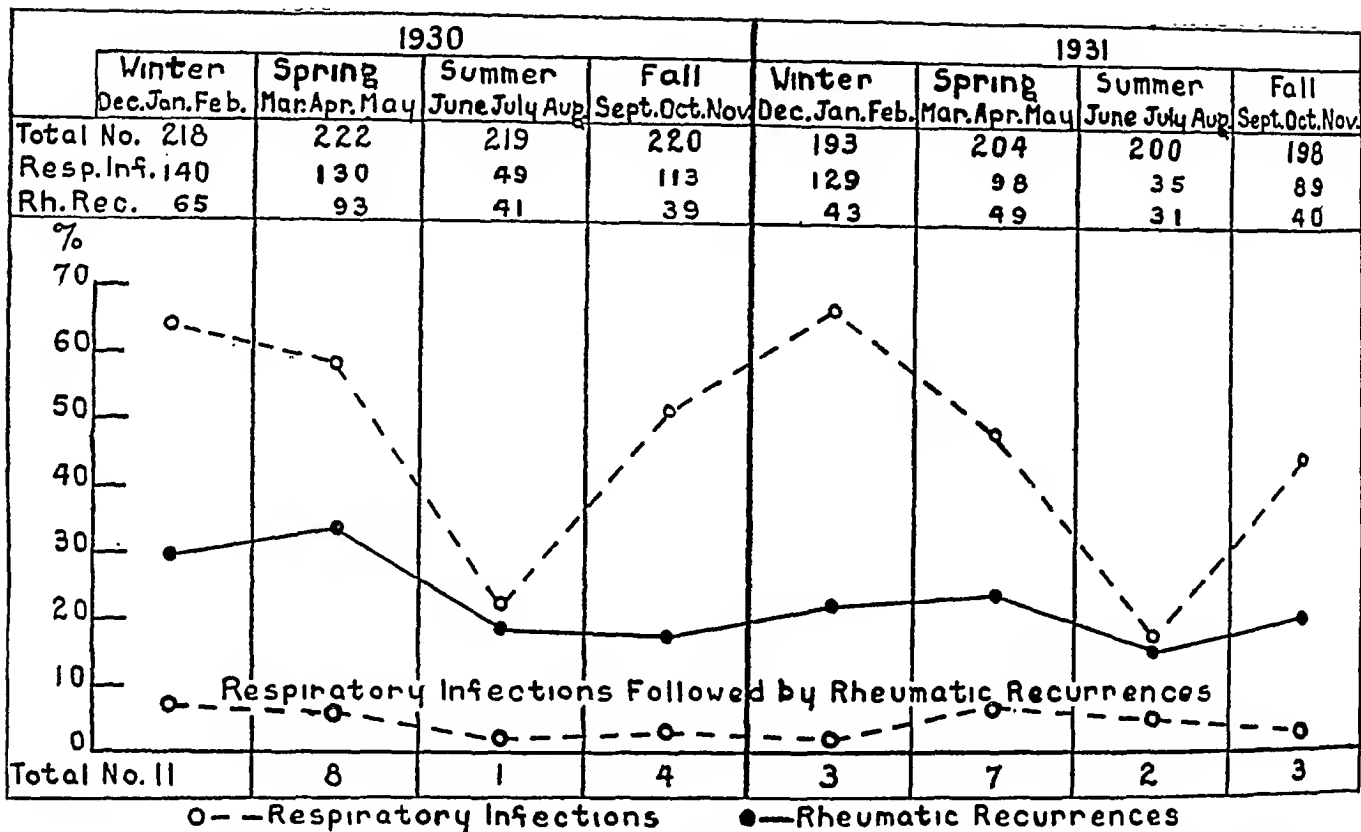


CHART 1. SEASONAL INCIDENCE OF RHEUMATIC RECURRENCES AND RESPIRATORY INFECTIONS 1930-1931

This chart illustrates the similar seasonal incidence of respiratory and rheumatic infections for 222 ambulatory rheumatic subjects.

In view of the etiological significance attributed to the presence of hemolytic streptococci in the flora of the throat during respiratory infections, it was considered worth while to include bacteriological and immunological observations in the following investigations.

To evaluate the possible relation of respiratory infections to rheumatic fever, the family was taken as a unit for study. Ninety-two families including 123 rheumatic children, members of the Cardiac Clinic, were selected for this study. These families have been under our observation for a period of two to fourteen years. Since September 1932 all the children attended the New York Hospital. About one-half of the rheumatic subjects are included in the 1930-1931 study.

Information as to the incidence and *time relationship* of respiratory and rheumatic attacks was obtained as accurately as possible. Trained investigators visited the homes weekly and recorded upon a family chart the symptoms of the illness of every member of the household. When necessary, physicians visited the homes. Weekly throat swabs for cultures were taken routinely from all rheumatic children, from a series of controls and

from every available member of the household during illness. Children visited the clinic for examination at least monthly. The majority of the members of the household were examined during the year. Observations were continued on children admitted to the pavilions and convalescent cottages of the New York Hospital during the period of study.<sup>2</sup>

A more detailed discussion of the bacteriological technique and criteria followed in these studies is included in the third paper of this series (7). Throat culture specimens were taken at regular weekly intervals, in some instances more frequently. After inspection of the pharynx, the surface was rubbed with a sterile dry cotton swab. Blood agar plates were inoculated and incubated for twenty-four hours. The relative number of each type of colony was estimated and recorded numerically from 0 to 4. The relative number of colonies of hemolytic streptococci isolated from the pharyngeal flora of each subject

<sup>2</sup> We wish to acknowledge our indebtedness to the resident staff of the New York Hospital and to Miss Marie L. Troup, Superintendent of the New York Hospital Convalescent Cottages.

was recorded and correlated with the clinical signs and symptoms.

The following rheumatic manifestations were recognized as evidence of activity: active carditis, chorea, arthritis, growing pains, pains in the joints, eruptions of the skin and rheumatic nodules.

Respiratory infections included the common cold, nasopharyngitis, tonsillitis, otitis, cervical

throat and the high incidence of respiratory infections in contrast to the insignificant rise of rheumatic recurrences during the months of March, April and May.

During the year, 98 per cent of the subjects experienced a total of 649 respiratory infections. About one-third of the respiratory infections were associated with either marked local or constitutional symptoms, the remaining were common

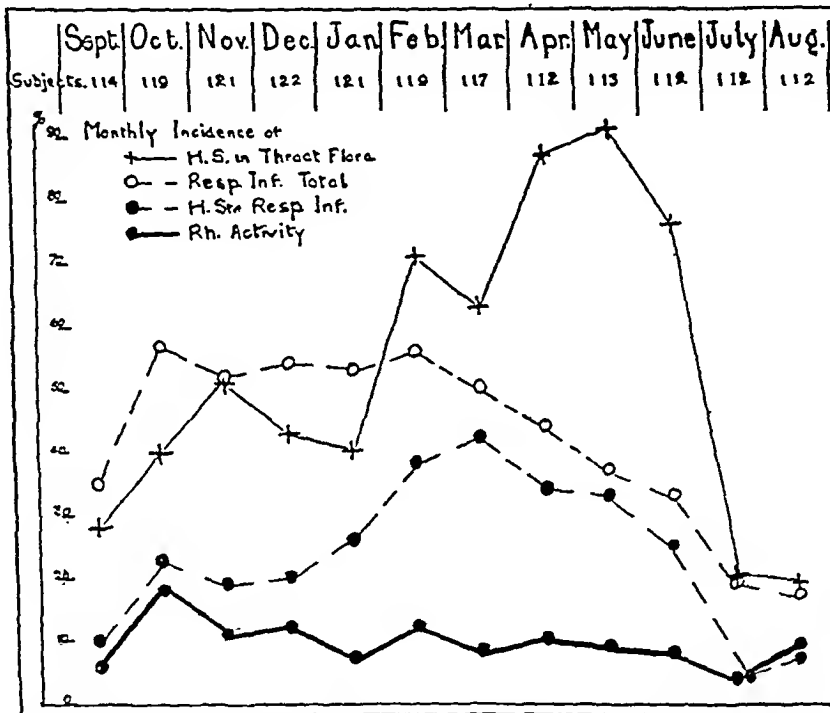


CHART 2. MONTHLY INCIDENCE OF RHEUMATIC RECURRENCES AND RESPIRATORY INFECTIONS EXPERIENCED BY 123 RHEUMATIC SUBJECTS 1933-1934

adenitis and bronchitis. When such infections were associated with the presence of hemolytic streptococci in the pharyngeal flora, they were designated as "*streptococcal*" respiratory infections for brevity. This, we believe, is in accord with the criteria apparently accepted in comparable investigations, although contrary to our own conception.

A comparison of the monthly incidence of respiratory infections and rheumatic recurrences experienced by the 123 rheumatic subjects over a period of twelve months, from September 1933 to September 1934, is graphically presented in Chart 2. Of particular interest is the increased incidence of hemolytic streptococci in the flora of the

colds. Many children suffered from persistent ethmoiditis and sinusitis during the period of observation. Three hundred and fifty-three (54 per cent) of the total respiratory infections were associated with hemolytic streptococci in the pharyngeal flora. In about one-third hemolytic streptococci predominated in the pharyngeal flora.

The designation, *streptococcal respiratory infection*, judging from the recent literature, is solely a bacteriological distinction and does not appear to be based on any diagnostic local signs or symptoms in the majority of instances. That such a bacteriological differentiation of respiratory infections would not appear to be a valid one, is indicated by the following. During the months

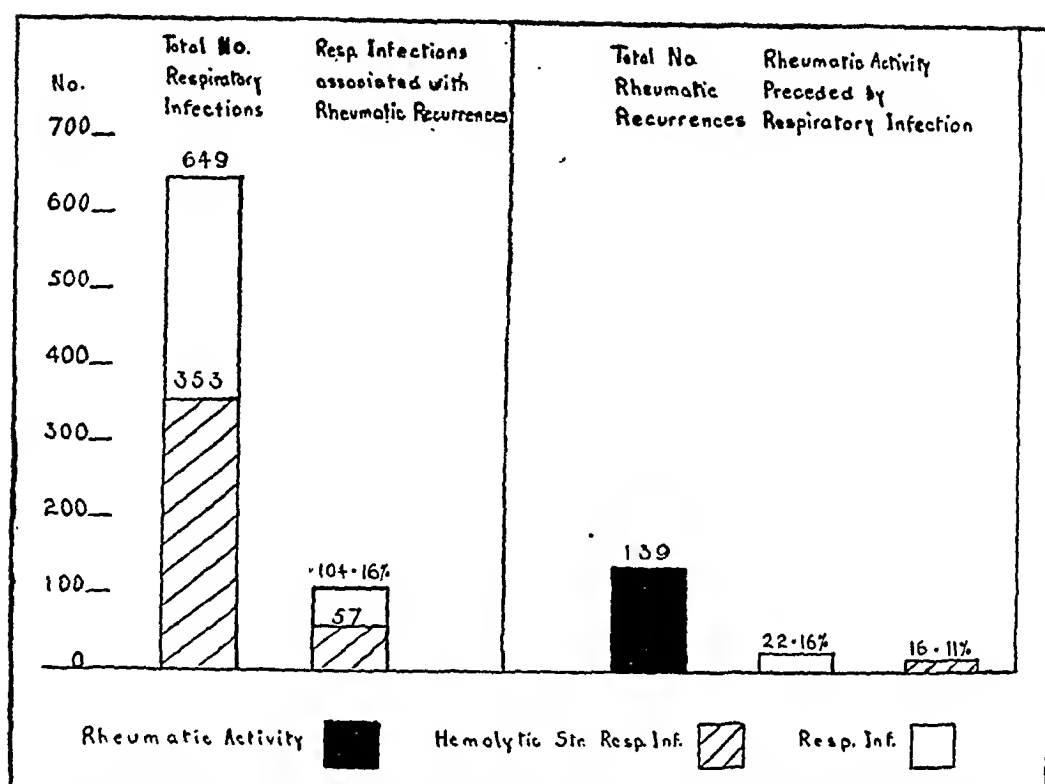


CHART 3. RELATIVE INCIDENCE OF RESPIRATORY INFECTIONS AND RHEUMATIC RECURRENCES 1933-1934 (123 RHEUMATIC SUBJECTS)

of March, April and May, hemolytic streptococci appeared in the pharyngeal flora in the majority of the subjects. It is significant that during this period hemolytic streptococci predominated in the pharyngeal flora of 50 per cent of the subjects who were well, 40 per cent during respiratory infections and 10 per cent during rheumatic activity. It is of interest that the majority of these respiratory infections were the common cold.

In marked contrast to the prevalence of respiratory infections during the year was the relatively low incidence of rheumatic recurrences.

Of the total 649 respiratory attacks only 16 per cent were associated with rheumatic activity. There did not seem to be any direct relation between the pharyngeal flora, or the type or severity of the respiratory infection and the rheumatic activity that happened to follow such infection. It is perhaps of some significance that during these observations the majority (84 per cent) of the respiratory infections experienced by these rheumatic subjects were not associated with rheumatic activity.

Sixty-one subjects (49 per cent) experienced

TABLE I  
*Relation of rheumatic activity to respiratory infection*

Rheumatic activity	Total number of cases	Preceded by		During respiratory infection		Not associated with respiratory infection		Classification doubtful
		"Streptococcal" respiratory infection	Non-streptococcal respiratory infection	"Streptococcal" respiratory infection	Non-streptococcal respiratory infection	Preceded by streptococci in throat	Negative	
Carditis, arthritis, nodules	8	0	0	4	2	1	1	
Arthritis	9	2	1	3	1	0	2	
Chorea	16	1	2	5	3	2	3	
Skin (purpura, urticaria)	6	2	0	2	0	1	0	1
Joint pains	100	11	19	27	19	13	11	
Total cases	139	16	22	41	25	17	17	1
Per cent		11.5	16	29	18	12	12	

139 rheumatic symptoms; about three-fourths, 100, were recurring joint pains, 6 purpura, 16 chorea, 9 arthritis and 8 carditis, arthritis and nodules. Analysis of the time relationship between these rheumatic recurrences and respiratory infections, summarized in Table I, is of interest.

month before the onset of rheumatic activity. The intervening period of apparent health was designated as the *quiescent interval*. Sixteen per cent of the rheumatic episodes were preceded by a respiratory infection (unassociated with hemolytic streptococci in the flora of the throat) and only 11.5 per cent of the 139 rheumatic recur-

1933-1934

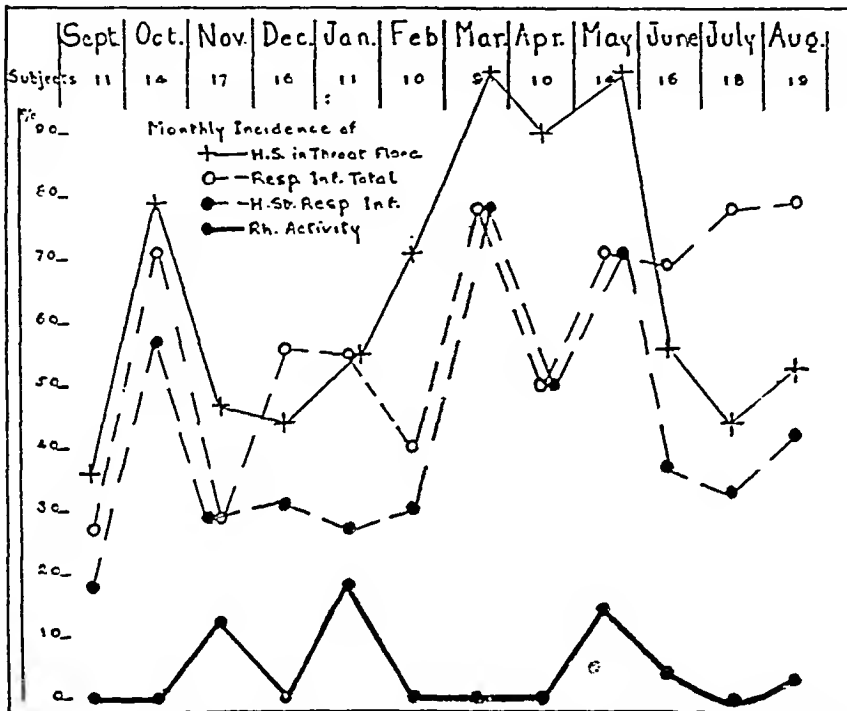


CHART 4. MONTHLY INCIDENCE OF RHEUMATIC RECURRENCES AND RESPIRATORY INFECTIONS EXPERIENCED BY 62 RHEUMATIC SUBJECTS AT THE NEW YORK HOSPITAL CONVALESCENT COTTAGES.

One-fourth (24 per cent) of the 139 rheumatic episodes were unrelated to respiratory infections. About one-half (47 per cent) of the rheumatic recurrences occurred either simultaneously with a respiratory infection or more frequently during a chronic respiratory infection, such as persistent ethmoiditis so commonly present in children. In some instances the respiratory infection occurred during the course of the rheumatic activity.

Of particular interest is a consideration of the incidence of rheumatic recurrences which were preceded (within a month) by a respiratory infection. Respiratory infections were considered to precede rheumatic activity if the termination of the respiratory infection was within a period of a

rences were preceded by a "streptococcal" respiratory infection. The quiescent interval subsequent to respiratory infections was infrequently observed to precede rheumatic activity. This is not in accord with the observations of other investigators.

In children it is particularly difficult to determine the onset of rheumatic disease when respiratory symptoms are present. Fever is frequently the only early objective symptom of rheumatic activity. It is suggested that in such cases rheumatic fever may be present at the onset of what appears to be a respiratory infection, although not easily detected clinically, and that the quiescent period may then represent the apparent

symptomless period, so commonly observed during the course of rheumatic activity (see Protocol J. V., Paper II of this series).

The occurrence of a crop of nodules, a sharp rise of temperature or a pericardial rub, following an apparently symptomless period of a week or ten days, is a characteristic train of symptoms during the course of rheumatic activity in children, unassociated with respiratory infections. Such episodes frequently lead one to attribute erroneously etiological significance to events, or to therapeutic or diagnostic measures, just preceding the appearance of these objective symptoms.

A consideration of the relation between respiratory infections in the household and the occurrence of rheumatic fever is of interest. Complete data for analysis were obtained for 79 rheumatic families comprising 117 rheumatic individuals under sixteen years of age, 164 non-rheumatic children and 183 adults. The monthly incidence of household infections for ten months (September to July) was as follows: two or more members per month experienced respiratory infections 193 times, respiratory infections with streptococci in the pharyngeal flora 102 times, and carried hemolytic streptococci in the throat 106 times (in apparent good health). During this period 50 children experienced 106 rheumatic recurrences. These rheumatic episodes did not appear directly related to the presence of household infections, occurring as frequently in the absence of such respiratory infections. It is perhaps of some significance that rheumatic subjects experienced an average of five respiratory attacks compared with three in the control group for the ten month (September to July) period.

During this investigation a series of 62 rheumatic children who were under daily observation at the New York Hospital Convalescent Cottages for a period of from two months to one year gave an opportunity for a more accurate study of the relation of respiratory infections to rheumatic fever in children living in close contact. The majority of the subjects were convalescing from severe rheumatic recrudescences, many were completely at rest in bed, others were ambulatory. Non-rheumatic convalescent children were also under the same supervision, and no attempt was made to isolate the rheumatic child. During the period of observations 105 respiratory infections,

of which 53 were accompanied by hemolytic streptococci in the pharyngeal flora, were experienced by the rheumatic children. Six subjects suffered nine rheumatic recurrences; three of the rheumatic episodes were preceded by "streptococcal" respiratory infections. Although three severe epidemics of respiratory infections associated with a predominance of hemolytic streptococci in the pharyngeal flora (October, March, May) were observed among these susceptible rheumatic subjects, there was no appreciable increase in the incidence of rheumatic activity during this period such as might have been expected from the reported epidemics in institutions under similar circumstances.

It is significant that during these various observations, although the majority (98 per cent) of the rheumatic children experienced one or more respiratory infections, only 16 per cent of these infections were associated with rheumatic activity. About one-half (49 per cent) of the children suffered one or more rheumatic recurrences, but only 11.5 per cent of the rheumatic episodes were preceded by "streptococcal" respiratory infection.

#### COMMENT

This investigation was conducted over a period of several years, comprising studies of a large group of rheumatic children observed in the homes, hospital wards and convalescent cottages. These studies are comparable to those reported by the majority of investigators with the exception that our investigations were limited to children.

It is evident from the data that our observations do not give support to the conception of a specific etiological relationship between respiratory infections and rheumatic fever. Their association would seem almost inevitable because of the observed frequency of respiratory infection in children. It is also possible that the rheumatic child is constitutionally vulnerable to both infections. This possibility is indicated by the frequency of a simultaneous onset of rheumatic activity and respiratory infection (following a common exciting factor). It is also suggested by the greater susceptibility of the rheumatic child to respiratory infection compared with that of a non-rheumatic brother or sister.

Although it is generally well recognized that the organisms isolated from throat cultures do not represent an accurate picture of the pharyngeal flora, merely indicating the fluctuation in the relative numbers of the various organisms rather than their presence or absence, recent investigations tend to ascribe etiological significance to the isolation of hemolytic streptococci from the flora during respiratory infections. Emphasis seems to be placed on the relative numbers of colonies of hemolytic streptococci present rather than on the local signs or symptoms.

Our observations would tend to minimize the diagnostic significance of the presence or absence of hemolytic streptococci in the pharyngeal flora. It was found that the relative numbers of these organisms in repeated consecutive cultures varied from predominance to few or none. The specificity of "streptococcal" respiratory infections based solely upon bacteriological criteria does not appear convincing in view of the observed comparable carrier rate of hemolytic streptococci in the pharyngeal flora of sick and well children. The immunological studies reported in the second paper of this series, showing a rise in the titre of antistreptolysin following respiratory infections unassociated with hemolytic streptococci in the pharyngeal flora is pertinent.

It is of interest to note in passing that since tonsillectomy has become an early routine procedure in children, tonsillitis is observed infrequently in the course of rheumatic disease. Recent investigators tend to substitute in its place nasopharyngitis.

The etiology of the rheumatic sequelae following scarlet fever is still controversial. Of interest is the evidence of Paul and Salinger (8) in their studies of the relation of rheumatic sequelae to scarlet fever that "like respiratory infections, scarlet fever activates the rheumatic process in the rheumatic child."

Wilson, Lingg and Croxford (9) suggested that the common age and seasonal incidence of rheumatic fever and scarlet fever may be factors influencing their association. In their observations, scarlet fever was more frequently followed by rheumatic recrudescences within the age period of five to nine years. Seventy-five per cent of the rheumatic subjects developing scarlet fever at other age periods did not have rheumatic sequelae.

During these observations (as can be seen in Protocol 4 (10)) the occurrence of scarlet fever during a severe carditis did not appear to influence the course of the latter.

While it is appreciated that the occurrence of respiratory infections in a rheumatic child may not be a fortuitous event, it would seem from our observations to bear no more specific etiological relationship to rheumatic disease than would be attributed to similar episodes occurring in the tuberculous child.

#### SUMMARY

1. There are presented investigations conducted over a period of several years comprising epidemiological, bacteriological and clinical studies of a large group of rheumatic children, observed in the homes, hospital wards and convalescent cottages.

2. Two hundred and twenty-two ambulatory rheumatic subjects five to fifteen years of age experienced 783 respiratory infections and 401 rheumatic recurrences (for a two-year period of observation 1930 to 1932). Less than 10 per cent of the rheumatic attacks were preceded within three weeks by a respiratory infection.

3. Of a total of 123 rheumatic subjects under close observation for twelve months, September 1933 to September 1934, 98 per cent suffered 649 respiratory attacks; of which 353 were associated with the presence of hemolytic streptococci in the throat flora. Eighty-four per cent of the respiratory infections were not associated with rheumatic activity.

4. Forty-nine per cent of the subjects experienced 139 rheumatic episodes; a *quiescent interval*, subsequent to "streptococcal" respiratory infection, preceded 11.5 per cent of the rheumatic episodes.

5. Sixty-two rheumatic subjects were under daily observation at the Convalescent Cottages for a two to twelve month period. During three epidemics of respiratory infection associated with a predominance of hemolytic streptococci in the pharyngeal flora, there was no appreciable increase of rheumatic activity.

6. Rheumatic subjects experienced an average of five respiratory attacks compared with an average of three for the non-rheumatic children of their respective households.



7. During the spring of 1934 hemolytic streptococci appeared in the pharyngeal flora of the majority of the rheumatic and non-rheumatic subjects.

8. During this season of its highest incidence, hemolytic streptococci predominated in the pharyngeal flora of 50 per cent of the rheumatic subjects during health, 40 per cent during respiratory infections, and 10 per cent during rheumatic activity.

#### CONCLUSIONS

The evidence presented does not support the conception of a specific etiological relationship between respiratory infections and rheumatic fever in children.

Our observations would tend to minimize the diagnostic significance of the presence or absence of hemolytic streptococci in the pharyngeal flora during respiratory infections. The designation *streptococcal respiratory infection*, based solely on bacteriological findings, would not appear to be justified.

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# THE RELATION OF UPPER RESPIRATORY INFECTIONS TO RHEUMATIC FEVER IN CHILDREN

## II. ANTIHEMOLYSIN TITRES IN RESPIRATORY INFECTIONS AND THEIR SIGNIFICANCE IN RHEUMATIC FEVER IN CHILDREN <sup>1</sup>

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In the preceding article (1) evidence was presented which would not support the conception of a specific etiological relationship between respiratory infections and rheumatic fever in children. The designation, "streptococcal" respiratory infection, based solely on bacteriological findings, was questioned.

During these observations as to the relation between respiratory infections and rheumatic fever, an opportunity presented itself for obtaining information as to the immunological response of children experiencing respiratory infection and rheumatic recurrences. The serological method described by Todd (2) in 1932 for titrating antistreptolysin in the blood serum, by which a diagnosis of preceding hemolytic streptococcal infection may be made, was utilized.

From limited observations of normal individuals, mainly adults and others suffering from miscellaneous and streptococcal infections, Todd (3) and Coburn and Pauli (4) considered the normal range of antistreptolysin in serum to be from 5 to 100 units, and values of 200 or more as significant of preceding streptococcal infections. They also observed the antistreptolysin content to be consistently high during rheumatic fever and considered this as immunological evidence that the hemolytic streptococcus initiates the rheumatic process.

Myers and Keefer (5), however, obtained an average in normals of 200 units, with a somewhat higher average, 500 units, in rheumatic fever, but they did not attribute etiological significance to this finding.

Wilson, Wheeler and Leask (6), in a preliminary report on the antistreptolysin content of the

serum in children, found that this antibody apparently passes through the placenta, being present in new-born infants. An analysis of the average titres for various age groups indicated a definite age correlation, the lowest values being obtained in the age period between three months and three years. It is of interest that the values obtained from sixteen mothers was within the normal range reported by Todd (3), but was significantly lower than those obtained for children six to sixteen years of age by these observers. The possible significance of this difference will be considered later.

Early in our studies it was apparent that single determinations of antistreptolysin were of little value because of the wide normal range and frequent presence of elevated titres for prolonged periods of a year or more. It was, therefore, considered advisable to obtain serial specimens at regular frequent intervals during the entire period of the observations. By this procedure it was possible to correlate the clinical course with the bacteriological and immunological findings of a representative series of subjects.

### SUBJECTS AND METHOD

Todd's method for determining antistreptolysin in the blood serum was followed. The test is based on the presence in the blood serum of an agent which is capable of combining with, and neutralizing, the hemolysin in filtrates of broth cultures of hemolytic streptococcus. Todd (2) prescribed the following standard units:

Red blood cells (rabbit)—0.5 cc. of a 5 per cent suspension of washed cells

Antigen-Streptolysin-Minimum hemolytic dose (M. H. D.). Smallest quantity of streptolysin filtrate that will completely hemolyze one unit of rabbit cells in one hour at 37° C. This is determined by titration and is expressed in cc.

<sup>1</sup> This study was conducted under a special grant from the Commonwealth Fund.

Antibody-Antistreptolysin-Neutralizing dose (N. D.). Smallest quantity of patient's serum, inactivated 15 minutes at 55° C., that will combine with and completely neutralize 2.5 units of streptolysin in 15 minutes at 37° C. This is determined by titration and is expressed in ordinary or decimal fractions of a cc. or in Todd units (3), which are the reciprocals of the decimal fractions and indicate the number of neutralizing doses in 1 cc. of serum.

The methods for the preparation of these reagents are given in detail in the appendix.

By adding diminishing quantities of serum to tubes containing 2.5 units of streptolysin, allowing 15 minutes at 37° C. for neutralization to take place, then adding one unit of red blood cells to each tube and incubating for one hour, the smallest amount of serum that would completely inhibit hemolysis was determined. This was recorded as the titre of the serum.

Adequate data for analysis was obtained for eighty rheumatic subjects who were under close observation for a period of from twelve to eighteen months. From serial antistreptolysin determinations, the basal level was obtained for each child. The lowest titre during the period of observation, which was maintained in apparent health, was selected as the basal level. In the majority of instances, periods of two to six months free from symptoms of infection preceded this value. The rise of titre above the basal level or above the level maintained during apparent health before the occurrence of respiratory infection or rheumatic activity was calculated.

A respiratory infection was considered a "streptococcal" infection if hemolytic streptococci were present in the pharyngeal flora during the respiratory infection, in contra-distinction to respiratory infections unassociated with hemolytic streptococci in the flora of the throat. Respiratory infections were graded as mild or severe based on local and constitutional symptoms.

The following were considered as rheumatic recrudescences: joint pains, purpura, arthritis, nodules, chorea and carditis.

#### OBSERVATIONS

In view of the observed frequency of respiratory infection and the high seasonal carrier rate of hemolytic streptococci in the pharyngeal flora of these children, it was obviously impossible to obtain antistreptolysin determinations in children free from either during the entire period of the

observations. In this study the basal values obtained for 80 rheumatic subjects during apparent health showed a range of from 25 to 715 units with an average of 135 units. This is somewhat lower than that observed in our preliminary study (6). The opportunity for special selection of subjects and the longer period of observation probably accounts for this difference. The antistreptolysin titre remained remarkably constant at various levels during periods when the subjects were free from respiratory infection. It is possible that the high basal levels which were maintained by apparently healthy children may represent chronic unrecognized infection or some infection occurring previous to our observation, as it was obviously impossible to eliminate subjects who had had respiratory infection the previous year. It would seem reasonable to consider these observations on the antistreptolysin content of the serum as representative for inactive rheumatic children, six to sixteen years old, in apparent health, although possibly not comparable to normal values given for adults.

A comparison of the range of antistreptolysin titre, the average titre and the rise observed during apparent health and infection is of interest. There was no significant difference in the range of antistreptolysin titre observed in subjects *during health* and *during respiratory and rheumatic infections*. There was no appreciable difference in the average titre of antistreptolysin obtained for subjects during respiratory infections not associated with hemolytic streptococci and similar infections associated with hemolytic streptococci in the pharyngeal flora.

*Following* respiratory infections irrespective of the bacteriological findings, a rise in titre was consistently although not invariably observed. The average rise and the percentage showing rise in titre was slightly higher following respiratory infections not associated with hemolytic streptococci in the pharyngeal flora, than following similar infections thus associated. The contrary might have been expected. Greater increments and higher percentage of subjects exhibiting a rise in titre were observed following severe respiratory and "streptococcal" infections than mild ones. The degree of rise was not always directly related to the severity of the respiratory infection or to the bacteriological findings. There was consider-

TABLE 1

*Antistreptolysin titres in respiratory infections, their significance in rheumatic fever in children*

		Basal values	Respiratory infection				"Streptococcal" respiratory infection				Not associated with respiratory infection		
			Mild		Severe		Mild		Severe		Hemolytic streptococcus in throat culture		Negative throat culture
			I*	A*	I	A	I	A	I	A	I	A	A
Number of cases		80	30	17	10	6	45	22	57	20	39	11	11
Antistreptolysin	Range	25 715	71 830	25 250	71 500	200 1000	33 830	50 333	50 620	25 1600	50 333	62 830	55 333
	Average	135	244	130	254	366	171	175	198	415	165	262	163
Cases with rise above basal level	Number		19	8	7	4	19	7	29	16	19	4	4
	Per cent		63	47	70	67	42	32	51	80	49	36	36
Rise in units per cc.	Range		0 300	0 130	0 360	0 900	0 133	0 100	0 300	0 1250	0 200	0 200	0 200
	Average		85	55	152	210	45	49	97	212	58	140	65

\* I = inactive rheumatic.

A = active rheumatic.

able individual variation in the degree of serological response to what appeared to be a common source of hemolytic streptococcal infection.

It is of passing interest that about 50 per cent of the subjects carrying hemolytic streptococci in the throat during apparent health exhibited a slight rise in antistreptolysin titre.

The low average antistreptolysin titres observed by us (in preliminary studies) in average normal infants and children under the age of five years and by Todd (3), Coburn and Pauli (4) in adults may be related to the relative infrequency of respiratory infections at these age levels compared with the observed high incidence of respiratory infections in children five to sixteen years of age.

Following respiratory infections, the antistreptolysin curve was characterized by a step-like elevation within one to three weeks of the onset, rising to a peak and falling either by lysis within one to two months or remaining plateau-like at intermediate levels for longer periods, showing successive peaks following repeated respiratory infections.

The findings in rheumatic subjects during inactive periods and during respiratory infection must be considered in evaluating the significance of the

antistreptolysin content of the serum during rheumatic activity.

Two-thirds of the subjects experiencing rheumatic recrudescences, unassociated with respiratory infections, did not show a rise of antistreptolysin titre. The average values obtained during rheumatic activity associated with respiratory infection and "streptococcal" respiratory infection were significantly elevated, but are comparable to values obtained in inactive rheumatic subjects during similar infections. The slightly higher average values, which obtained in the group of active rheumatic subjects who experienced severe "streptococcal" respiratory infection, were influenced by the occurrence in five subjects of extremely high antistreptolysin values during intercurrent severe streptococcal infection (see protocol of Case 4, C. V.).

The rise and level of antistreptolysin in serum following respiratory infections seemed closely related to the extent of the constitutional and local symptoms associated with the respiratory infection, irrespective of the presence of hemolytic streptococci in the flora of the throat.

The antistreptolysin curves observed in subjects who developed respiratory infections either simul-

taneously with, or during, rheumatic activity were similar to those described in subjects experiencing respiratory infections alone and bore no relation to the clinical course of rheumatic activity.

Antistreptolysin curves observed during rheumatic activity either maintained the same level noted before the onset of rheumatic activity or assumed the trend of curves observed during respiratory infections. A sharp rise of titre at the onset of rheumatic activity, unaccompanied by respiratory infection, which has been described by Coburn and Pauli, was not obtained. High titres during rheumatic activity were observed in subjects who experienced simultaneously rheumatic activity and respiratory infection. Other subjects maintained a high antistreptolysin level during rheumatic activity which had been present previously for a period of months. A rise of titre was frequently noted during the course of rheumatic activity, following intercurrent respiratory infection, indicating the ability of the subject to react to streptococcal infection.

matic fever may be more clearly seen in the following protocols of representative cases.

#### PROTOCOLS

*Case 1, E. Mc.* Girl, six years of age, suffered from first attack of rheumatic infection (chorea) March 1933, from which she made an uneventful recovery in June. During the year of observation 1933-1934 she did not exhibit any evidence of rheumatic activity, although experiencing nine respiratory infections associated with the predominance of hemolytic streptococci in the flora of the throat on four occasions. She also exhibited, in December, a significant rise of antistreptolysin in the serum to 500 units, following a respiratory infection. This case illustrates the presence of "hemolytic streptococcal" infection in a susceptible rheumatic subject, unaccompanied by rheumatic activity.

*Case 2, V. B.* Girl, six years of age, had her first attack of rheumatic infection (arthritis) in May 1933, followed by an attack of chorea in June, lasting six weeks. In July, 1933, tonsillectomy was performed, and the child remained free from rheumatic activity until June 2, 1934, at which time she had a second attack of chorea. In October and November, 1933, she experienced two severe respiratory infections (unassociated with

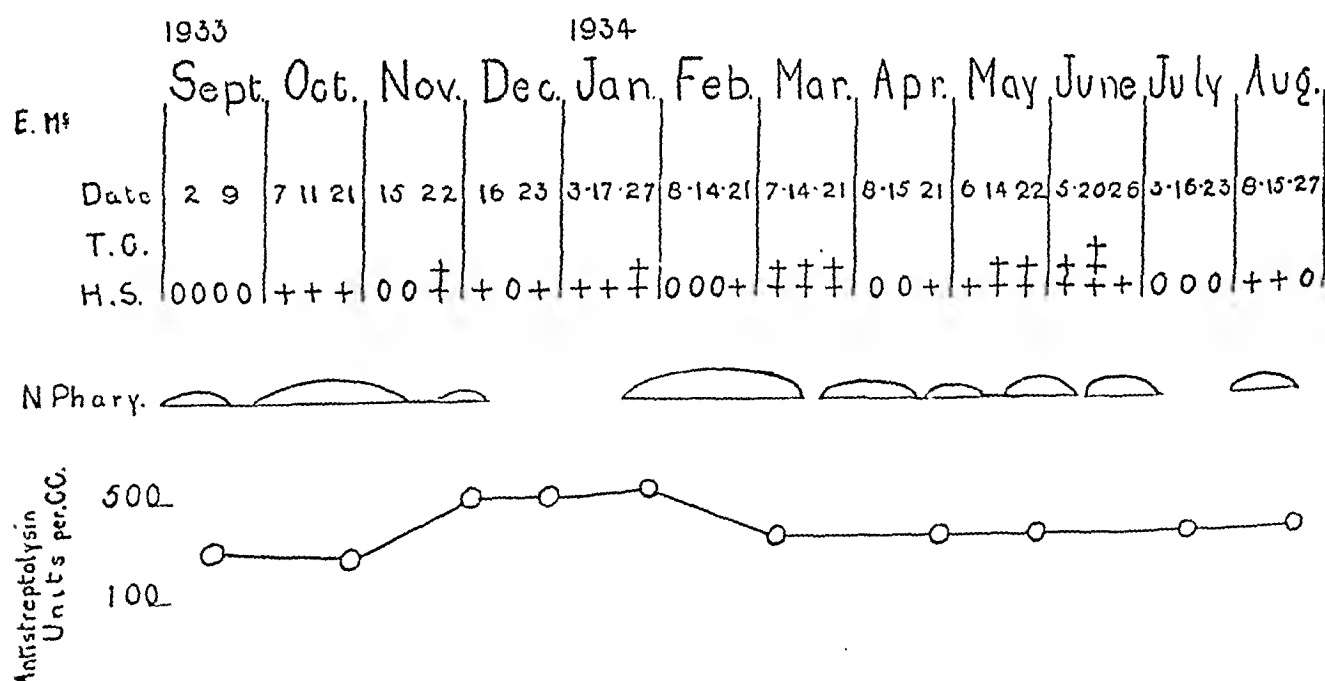


CHART 1. DATA ON CASE 1, E. Mc.

There did not appear to be any relation between the type of rheumatic activity and the antistreptolysin level. Low and high titres were observed in subjects during the last weeks of a fatal rheumatic carditis.

The relation between antistreptolysin content in the serum during respiratory infections and rheu-

hemolytic streptococci in the pharyngeal flora) which were followed by a significant rise of antistreptolysin titre, 615 units; and in February and March, 1934, three respiratory infections (with hemolytic streptococci in the throat) which were followed by a comparable increase in antistreptolysin. Rheumatic fever did not follow any of the respiratory infections. The chorea which occurred in June 1934 was preceded by two

	1933				1934							
V.B.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.
Date	27	3-11-21	17-23	6-9-16	10-7-24	3-7-21	4-11-28	3-7-21	6-13-19	16-13-23	13-20-30	20
T.G.												
H.S.	00	0000	0000	0000	+00	+0	++	++	0+0	00+0	0000	0

Resp. Inf.

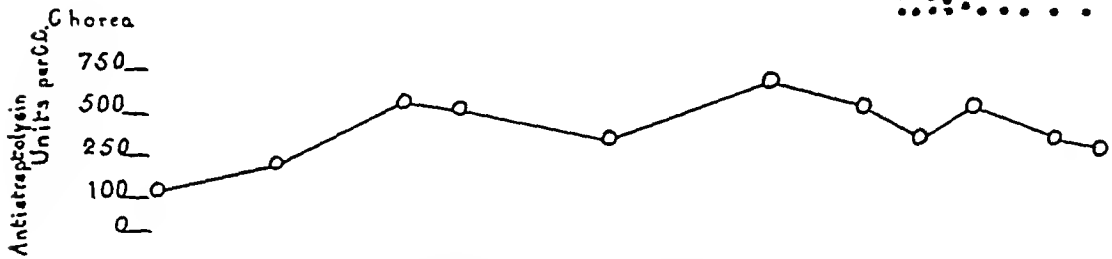
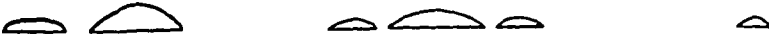


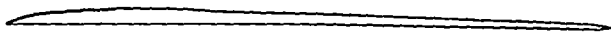
CHART 2. DATA ON CASE 2, V. B.

	1933				1934							
T.L.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.
Date	18	1-16-21	16-6-15	13-20-27	3-10-17	7-14-27	3-14-28	7-16-25	19-16-23	6-20-27	2-23-30	6-29
T.G.												
H.S.	00	0000	0000	0000	+000	0000	0000	0000	+0	0+0	+0+	00

N. Phary.



Sinusitis



Chorea

.....

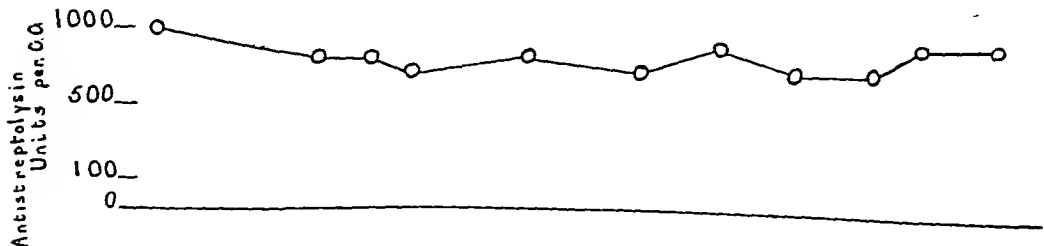


CHART 3. DATA ON CASE 3, T. L.

months of good health and was not accompanied by a significant rise in antistreptolysin titre. This case illustrates the rise of antistreptolysin in serum following a respiratory infection (non-streptococcal).

*Case 3, T. L. Boy*, ten years of age, had his onset of rheumatic disease (severe chorea) in September, 1932, from which he recovered in November, 1932. He had recurrent mild attacks of chorea in the summers of 1933 and 1934, of three weeks duration. In September, 1933, he was well and free from respiratory or rheumatic activity. At this time determination of the antistreptolysin content of the serum revealed a titre of

a recurrence (rheumatic arthritis and carditis). He remained at Convalescent Cottages from March 31, 1933, until September, 1933, when he returned to the clinic.

In the fall of 1933 hemolytic streptococci were absent from the pharyngeal flora. He suffered repeated respiratory infections coincident with joint pains. In the first week of December there occurred simultaneous symptoms of a respiratory infection and rheumatic fever characterized by joint pains, arthritis and carditis. He was admitted to the pavilion of the New York Hospital and while there, on February 18, developed scarlet fever during the height of a severe carditis accompanied by con-

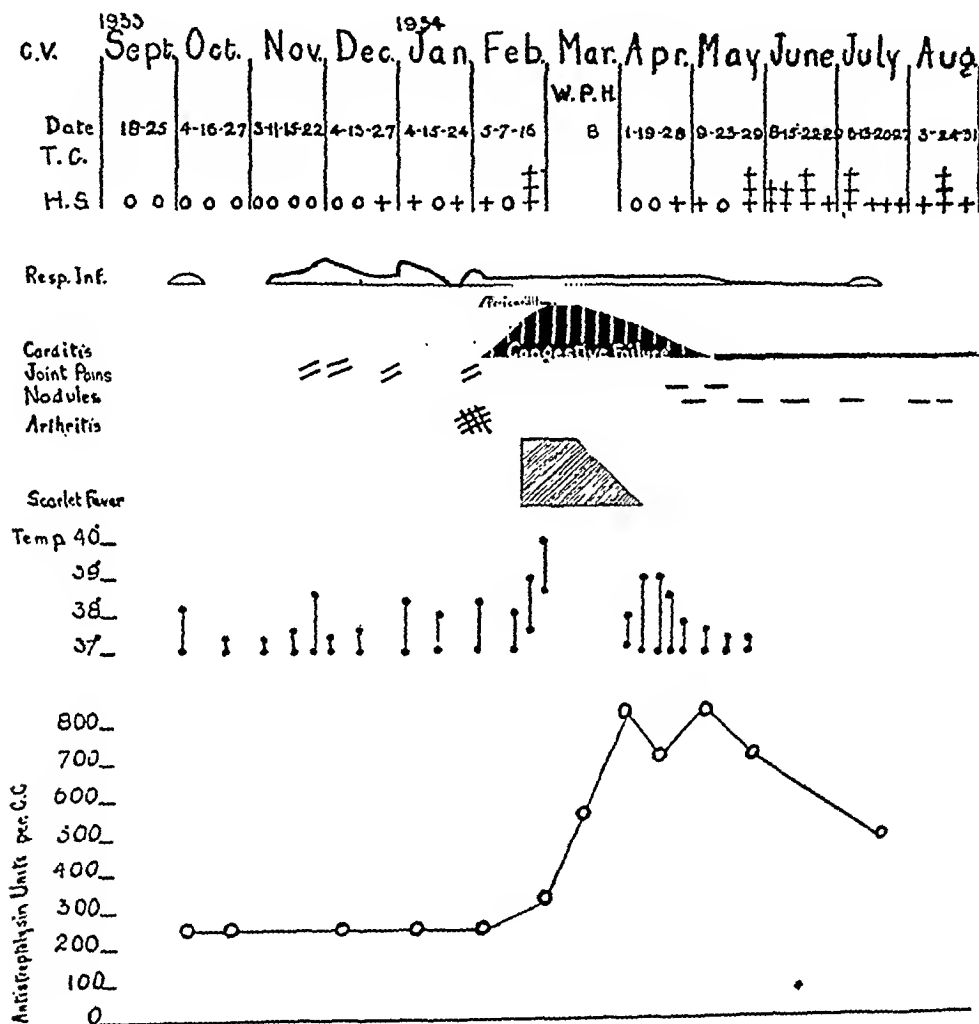


CHART 4. DATA ON CASE 4, C. V.

1,000 units; repeated examinations during the year gave values between 830 and 1,000 units. Hemolytic streptococci were not recovered from the pharyngeal flora until May, 1934. The occurrence of a sinusitis, following a respiratory infection, did not appear to influence the antistreptolysin titre. This case illustrates the persistent presence of high titre for a period of one year in a child during health, respiratory infection and rheumatic activity.

*Case 4, C. V. Boy*, six and a half years of age, had his onset of rheumatic activity (arthritis and chorea) at the age of three years. In March, 1933, he suffered

gestive failure. He was removed to Willard Parker Hospital for a six week period and returned to the New York Hospital, April 6. At that time there was still evidence of congestive cardiac failure and nodules.

Of particular interest is the antistreptolysin curve during this period. It can be seen in the accompanying graph, Chart 4, that the onset of rheumatic fever in December was not accompanied by a rise in antistreptolysin titre, while the occurrence of scarlet fever in February was followed by a significant rise in titre.

*Case 5, J. V. Boy* of fifteen years, who has been under our observation since he was eight years old, had

note that two-thirds of the total respiratory infections experienced by these 80 subjects were unassociated with rheumatic activity. Of the remaining third, two-thirds occurred either simultaneously with, or during, the rheumatic episode.

The wide normal range of antistreptolysin titre, the individual variation in response to streptococcal infection, the frequency of respiratory infections observed in these subjects, as well as the high carrier rate of hemolytic streptococci, make it difficult to evaluate their possible significance and relation to rheumatic fever. However, it would appear to be reasonable to state that our data do not indicate that the presence of hemolytic streptococci in the pharyngeal flora or that the presence in high titre of antistreptolysin in the serum is necessarily conclusive evidence of a streptococcal respiratory infection.

However, if we accept the criteria mentioned as bacteriological and immunological evidence of "streptococcal" respiratory infection, it is apparent from our observations that "streptococcal" respiratory infection did not, in the majority of cases, activate the rheumatic process and that a rise of antistreptolysin was not a constant accompaniment of rheumatic activity. Its presence appeared to be more frequently related to the coincidental occurrence of a respiratory infection.

Our observations being limited to children may not be entirely comparable to other investigations including adults. It may also be possible that the reaction to streptococcal infection in adults, like that to rheumatic fever, may be different from that in children.

#### SUMMARY

1. There is presented a correlation of the clinical course with the bacteriological and immunological observations in 80 rheumatic subjects observed over a period of twelve to eighteen months.

2. The antistreptolysin titre for rheumatic subjects during apparent health gave a basal average of 135 units with a range of 25 to 715 units.

3. There was no significant difference in the range of antistreptolysin titre observed for subjects during apparent health and during respiratory and rheumatic infection.

4. A comparison of the antistreptolysin titres of inactive rheumatic subjects during respiratory and "streptococcal" respiratory infections

showed a higher average titre and greater rise in titre for subjects experiencing respiratory infection unassociated with hemolytic streptococci in the pharyngeal flora.

5. Two-thirds of the subjects experiencing rheumatic activity unassociated with respiratory infections did not exhibit a rise in antistreptolysin titre.

6. The antistreptolysin titre of active rheumatic subjects experiencing respiratory and "streptococcal" respiratory infections was similar to that observed for inactive rheumatic subjects experiencing these infections.

7. Following respiratory infections the antistreptolysin curve was characterized by a step-like elevation within one to three weeks of the onset, rising to a peak and falling by lysis within one or two months or remaining plateau-like at intermediate levels for longer periods, showing successive peaks following repeated respiratory infections.

8. The rise of the level of antistreptolysin in the serum following respiratory infections seemed directly related to the extent of the local and constitutional symptoms irrespective of the presence of hemolytic streptococci in the pharyngeal flora.

9. The antistreptolysin curves observed in subjects who developed respiratory infection simultaneously with, or during, rheumatic activity were similar to those described in subjects experiencing respiratory infections alone and bore no relation to the clinical course of rheumatic activity.

#### CONCLUSION

1. These observations do not support the assumption that a rise in the antistreptolysin titre of the serum is conclusive evidence of streptococcal respiratory infection.

2. A rise in the antistreptolysin titre is not a necessary accompaniment of rheumatic fever in children.

#### APPENDIX

Todd stated in his original article (2) that a serum of known antistreptolysin titre was necessary as a control of the antigen-antibody reaction upon which this test is based. Hodge and Swift (7) proved experimentally that the minimum hemolytic dose is not a reliable unit for standardizing the streptolysin which is used as antigen. They demonstrated that under different conditions of age, oxidation, reduction and temperature a single streptolysin filtrate would show wide variations



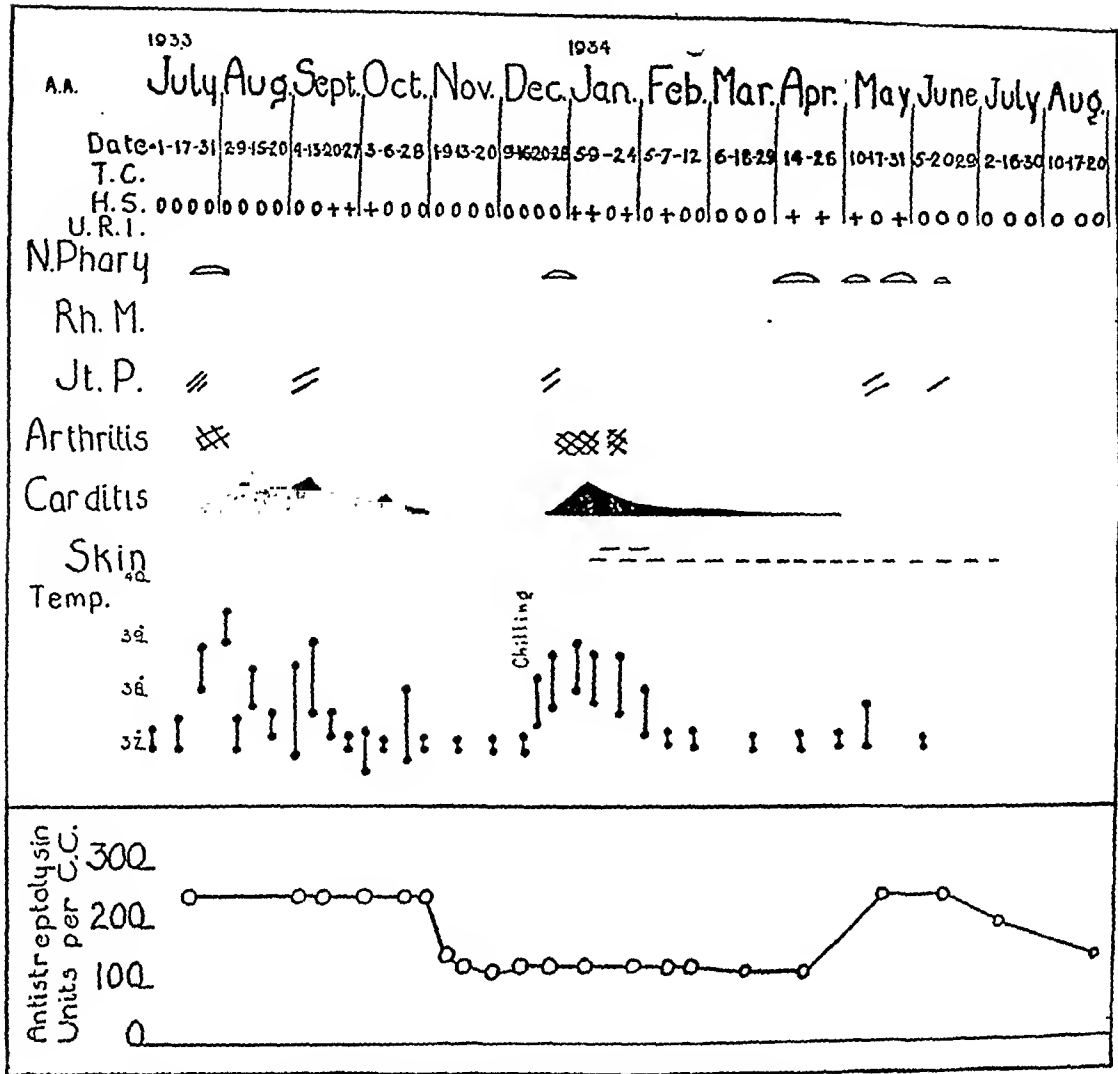


CHART 6. DATA ON CASE 6, A. A.

COMMENT

At the outset, it was arbitrarily assumed first, that respiratory infections associated with the presence of hemolytic streptococci in the flora of the throat were to be designated as "streptococcal" infections, and second, that a rise in the antistreptolysin titre of the serum was immunological evidence of a previous streptococcal infection. These criteria, we believe, are in accord with the statements of other observers in similar investigations.

The antistreptolysin content of the serum following such infections as scarlet fever, erysipelas and severe streptococcal respiratory infections associated with local and constitutional symptoms has been found to be significantly increased by all observers.

Of particular interest is the rise in titre in two-thirds of subjects following upper respiratory infections unassociated with hemolytic streptococci

in the pharyngeal flora. One must assume either that hemolytic streptococci were present although not found on examination of repeated throat cultures, or that the antistreptolysin content of the serum is not strictly type specific as Todd (3) believes, and that possibly partially hemolytic streptococci (alpha prime) predominant in the pharyngeal flora may play a rôle. Again, a rise in titre may be due to non-specific stimulation of this antibody during infection.

Irrespective of the significance of these findings, it is evident from our data that the antistreptolysin content of the serum during rheumatic activity appears to be related to the associated respiratory infection rather than to the rheumatic process. This view would seem to be supported by the low titres observed (in the necessarily limited number of observations) in subjects experiencing rheumatic activity unassociated with respiratory infection. It is also worthy of

note that two-thirds of the total respiratory infections experienced by these 80 subjects were unassociated with rheumatic activity. Of the remaining third, two-thirds occurred either simultaneously with, or during, the rheumatic episode.

The wide normal range of antistreptolysin titre, the individual variation in response to streptococcal infection, the frequency of respiratory infections observed in these subjects, as well as the high carrier rate of hemolytic streptococci, make it difficult to evaluate their possible significance and relation to rheumatic fever. However, it would appear to be reasonable to state that our data do not indicate that the presence of hemolytic streptococci in the pharyngeal flora or that the presence in high titre of antistreptolysin in the serum is necessarily conclusive evidence of a streptococcal respiratory infection.

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in the minimum hemolytic dose, yet in all instances, practically the same quantity of serum was required to effect complete inhibition of hemolysis. It was evident that under these conditions there were components in the streptolysin that would combine with antistreptolysin although they were not, themselves, hemolytic (partially oxidized streptolysin?). For this reason Hodge and Swift recommended that the combining power, which is stable, and not the hemolytic activity, which is variable, be used as the criterion in the standardization of streptolysin. This would necessitate the adoption of a standard serum of known antistreptolysin content against which all new lots of streptolysin could be titrated, and their combining doses determined. Todd has already made this practicable by his preparation of horse serum globulin, of high antistreptolysin titre, which is remarkably stable.

It appears that the factors involved in lysin-antilysin reactions are analogous to those concerned in toxin-antitoxin neutralization. In the latter instance no accurate standardization was possible until the neutralizing dose was replaced by the combining dose, and a standard serum of known antitoxin content was adopted.

### *Preparation of Reagents*

#### *Rabbit red blood cells:*

On the afternoon before the tests were to be made, blood was withdrawn from the rabbit's heart, placed in a sterile tube, defibrinated by stirring with wooden applicators and stored in refrigerator over night. Next morning the cells were washed six times with sterile 0.9 per cent sodium chloride solution. In the final washing they were centrifugalized 15 minutes at 1500 R.P.M.; they were then diluted to make a 5 per cent suspension.

#### *Streptolysin filtrate:*

The broth in which the streptococci were cultivated was prepared by a modification of the method described by Todd and Hewitt (8). Fresh, finely minced beef hearts, trimmed free of fat, were used instead of horse flesh; these were received from the abattoir the same day the animals were killed. Two liters of distilled water were added to each kilo of meat in an enamelware kettle, the mixture was well stirred and placed in refrigerator over night. Fat particles, if present, were skimmed from the surface, and the mixture was heated slowly over a free flame until the temperature reached 80° C., where it was kept for 30 minutes. The contents of the kettle were stirred constantly during the heating process.

The fluid was then strained through coarse wire gauze, filtered through Prat-Dumas number 45 filter paper and collected in a tall glass cylinder. Part of the residual fluid was removed from the meat by gentle pressure. The temperature of the infusion after filtration was about 50° C. The following ingredients were added: proteose-peptone, "Difco," 20 grams per liter, sprinkled on surface and allowed to dissolve; dextrose, sodium chloride, and sodium bicarbonate, 2 grams of each per liter; dibasic

sodium phosphate (12 H<sub>2</sub>O), 1 gram per liter. When these were dissolved, reaction was adjusted to pH 8.0 with 10 per cent sodium hydroxide solution. Medium was well stirred and placed in refrigerator over night.

A heavy amorphous sediment had formed, leaving a fairly clear supernatant fluid which was removed with a siphon and filtered through Whatman number 12 filter paper. Medium was sterilized by filtration through a battery of six Chamberland F bougies, using a water vacuum pump. The finished product was collected, in 1500 cc. lots, in two-liter flasks closed with two-hole rubber stoppers provided with two right angle glass tubes. The tube to which the suction was applied was filled with non-absorbent cotton to prevent air contamination; the other was covered with a small test tube when not in use. When filtration was completed, flasks were placed in incubator for two days as a sterility test.

Each 1500 cc. lot was inoculated with 2.5 cc. of an 8-hour culture of hemolytic streptococcus<sup>2</sup> in 0.3 per cent dextrose hormone broth. The inoculation was made through the open tube in the stopper of the flask while gentle suction was being applied to the other tube. After 16 hours incubation flasks were placed in ice water to check growth and culture was filtered through six Chamberland F bougies. The first 300 cc. of filtrate were discarded because of the low streptolysin content (8); the remainder was collected in 1500 cc. lots by the same procedure that was used with the broth filtrate. The open tube in the stopper was sealed off in a Bunsen flame, the other tube was connected with a water pump, and strong suction was applied for 2½ hours, the flasks being immersed in a water bath at 30° C. Bubbling was vigorous at first, but gradually diminished and finally ceased. Vacuum was released, 1.5 gram of sodium hydrosulphite (Baker) and 15 cc. of N/1 sodium hydroxide were added to each 1500 cc., and suction was again applied for 30 minutes. The reduced filtrate was then distributed in 50 cc. Florence flasks, sealed with a 4 cm. layer of sterile vaseline containing 12 per cent paraffin, and stored in refrigerator.

After three weeks the reaction of this filtrate was pH 7.3; the minimum hemolytic dose was 0.05 cc. (kept at 0° C., this showed no change in ten months).

#### *Antistreptolysin serum:*

Patient's blood was withdrawn with a dry sterile needle and syringe, placed in a sterile tube and allowed to clot. Tube was centrifugalized, serum was removed with a pipette and transferred to another tube which was then closed with a sterile paraffined cork and stored in refrigerator. Before testing the serum the tubes were placed in a water bath at 55° C. for 15 minutes to inactivate any natural hemolysin that might be present. Repeated determinations made with the same serum

<sup>2</sup> We used strain WPRL which we obtained through the kindness of Dr. Hodge of the Rockefeller Institute. This stock culture was kept in blood broth.

# THE RELATION OF UPPER RESPIRATORY INFECTIONS TO RHEUMATIC FEVER IN CHILDREN

## III. THE SEASONAL BACTERIAL FLORA OF THE THROAT IN RHEUMATIC AND NON-RHEUMATIC CHILDREN

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(Received for publication December 1, 1934)

In the preceding articles of this series the significance of hemolytic streptococci in the pharyngeal flora of rheumatic subjects has been discussed. In this paper a more detailed analysis of the bacterial flora of the throat in rheumatic and non-rheumatic children is presented.

Many investigators have studied the bacterial flora of the throat under various conditions of health and disease. Bloomfield (1) concluded from his results with six healthy individuals and a few others that a true picture of the normal pharyngeal flora is obtained only by making repeated cultures from the same individual. This procedure has been generally followed by most of the other workers in this field. Shibley, Hanger, and Dochez (2) made frequent cultures from thirteen individuals over a period of nine months. McCartney (3) reported the results of numerous routine throat examinations of healthy children. Fox and Stone (4), Meleney (5), Helmholz (6), and Bourn, Carpenter and McComb (7) were primarily interested in the occurrence of hemolytic streptococci in the throat.

These researches and many others establish the fact that there is a basic flora of the throat which remains fairly constant for each individual and contains non-hemolytic streptococci, gram-negative cocci of the catarrhalis, *pharyngis siccus*, or chromogenic types, *Staphylococcus albus*, or diphtheroids in various combinations. Hemolytic streptococci, pneumococci, *Staphylococcus aureus*, hemoglobinophilic organisms of the influenza or "X" types, Friedlaender's bacillus and others are frequently present as transient invaders.

Coburn (8) included an extensive survey of the situation with respect to throat cultures in his studies on rheumatic fever. He believed that the frequency of transient upper respiratory infection

with hemolytic streptococcus is the environmental factor of importance in the genesis of the rheumatic state.

Weinstein and Styron (9) studied throat cultures from 148 rheumatic patients and 173 persons who were normal or had diseases other than rheumatic fever. They reported approximately the same percentage of cultures positive for hemolytic streptococci in the two groups and found that "throat cultures of patients with rheumatic fever taken during an infection of the upper respiratory tract showed no greater incidence of hemolytic streptococci than those from other persons who were suffering from a cold or from a sore throat." The majority of their rheumatic patients who had exacerbations showed hemolytic streptococci in their throat cultures; they believe that this fact suggests a possible relationship between this organism and the reappearance of symptoms.

The growing tendency to regard the hemolytic streptococcus as an important, or even an essential, factor in the etiology of rheumatic fever led us to undertake the present investigation. The subjects were children and a few adolescents, rheumatic and non-rheumatic; no adults were included. The two principal objectives were: (a) to see if there is a significant difference between the incidence of hemolytic streptococcal invasion of the throat in rheumatic and in non-rheumatic children, and (b) to obtain information as to the relationship of such invasion to upper respiratory infection and to rheumatic activity. The frequency and time of appearance of other transient invaders were also noted.

The children under observation were seen in the pavilions and clinics of New York Hospital, at Convalescent Cottages, in school, or at their homes



from 109 non-rheumatic subjects. The percentage incidences, by months, of various organisms, are presented in Charts 1 and 2. In most individuals the basic flora of the throat was remarkably constant; transient invaders appeared frequently, but when they departed there was a rapid reversion to the original flora. The percentage

infected persons. Many of the positive cultures in both the rheumatic and control subjects came from children who presented no symptoms of hemolytic streptococcus infection and were apparently healthy. Throat cultures were recorded as positive when hemolytic streptococci were present, regardless of the number of colonies. It was

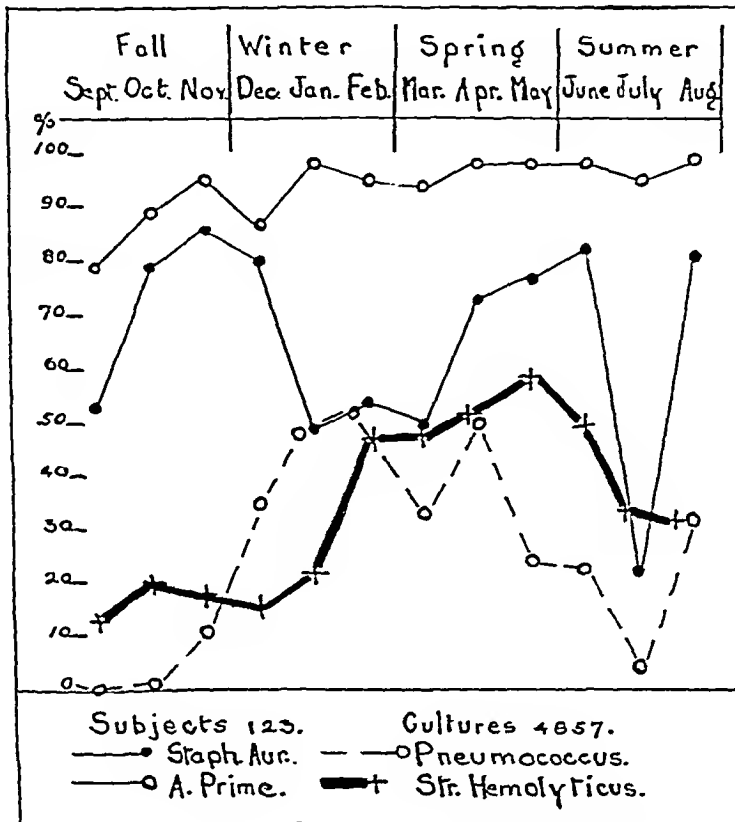


CHART 1. SEASONAL PHARYNGEAL FLORA IN RHEUMATIC SUBJECTS.\*

of cultures positive for hemolytic streptococci shows a striking parallelism in the two groups. The increased prevalence of these organisms in the throat during the winter and spring months, in this locality, has been noted by many observers, but there has been too little emphasis on the fact that this increase shows in carriers as well as in

found that the relative quantities of these organisms in repeated consecutive cultures varied from predominance to few or none. This was true for healthy carriers as well as for children with upper respiratory infections or with rheumatic activity. It seems evident, therefore, that etiologic significance can be attributed to an organism in the flora of the throat only when its occurrence in culture is associated with characteristic local signs and symptoms. Bacteriological evidence alone does not appear to be sufficient, since the findings in these cultures could not be correlated with the presence or severity of the local signs and symptoms. This point has been discussed in its rela-

\* Alpha and gamma streptococci, gram-negative cocci and other organisms normally present in the pharyngeal flora showed no significant seasonal variations in their incidence. The curves for the hemoglobinophilic and Friedlaender bacilli remained below the 20 per cent line throughout the year.

tionship to rheumatic activity in the first paper of this series.

Other organisms, *Staphylococcus aureus* and pneumococcus in particular, show a seasonal variation similar to that of the hemolytic streptococcus. In the routine diagnostic work of this and other laboratories it has been found that in recent

dren with and without clinical evidence of upper respiratory infection is presented in Chart 3. Two representative months, March and November, have been selected. Here it is shown that there is no significant difference in the incidence of hemolytic streptococci in throat cultures from the two groups; other months showed similar

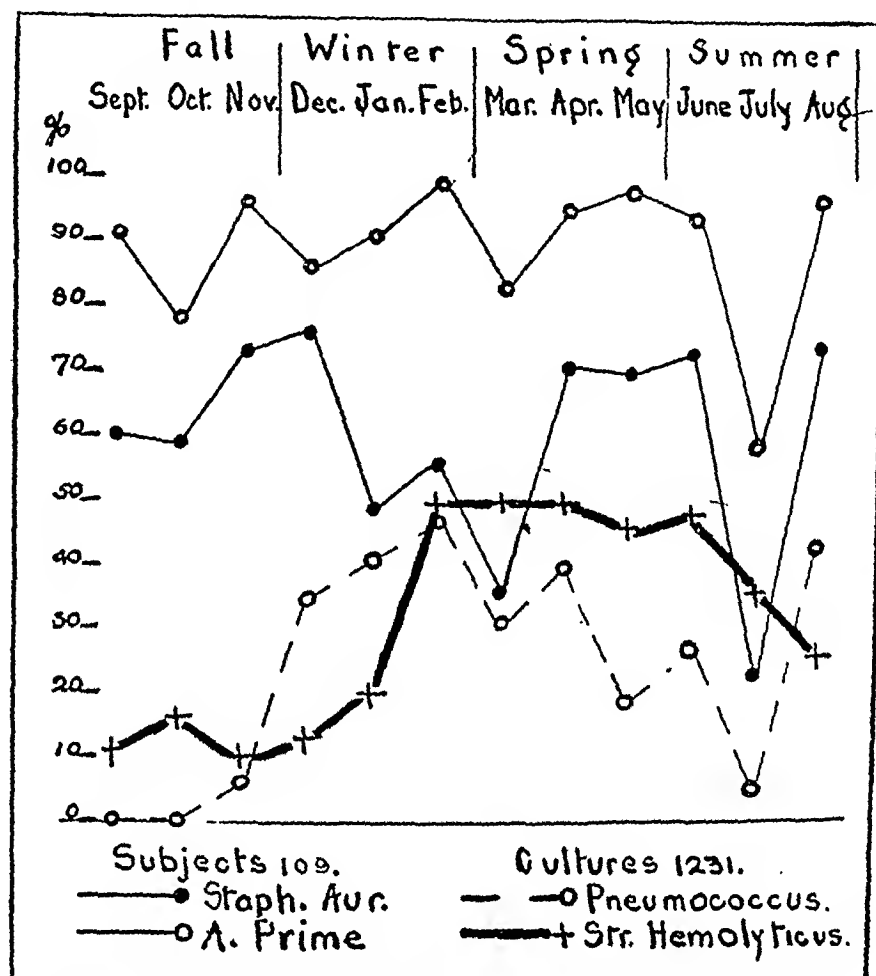


CHART 2. SEASONAL PHARYNGEAL FLORA IN CONTROL SUBJECTS.

years *Staphylococcus aureus* infections, occurring primarily or as complications of upper respiratory disease, were unusually prevalent during November and December. Pneumococcus lobar pneumonia shows its greatest incidence during the winter and early spring months. The periods of maximum frequency of these organisms in the throat coincide with the seasons in which they show their greatest clinical activity, yet none of these children with positive cultures had a detectable staphylococcus infection, and there was not a case of pneumococcus pneumonia in either group.

A comparison of the flora of the throat of chil-

percentages. There is no indication in these findings that upper respiratory infections are necessarily associated with any specific organism or group of organisms.

#### SUMMARY AND CONCLUSIONS

1. The data presented are based on a twelve-month study of 4867 throat cultures from 123 rheumatic children and 1231 cultures from 109 non-rheumatic children.

2. In addition to the basic flora of the throat, which is relatively constant for each individual, transient invaders are frequently found and tend

to show their maximum incidence at well-defined seasons of the year.

3. The seasonal incidence of various organisms in the pharyngeal flora must be considered in evaluating their possible etiological significance.

4. A comparison of throat cultures from rheumatic and non-rheumatic children shows no sig-

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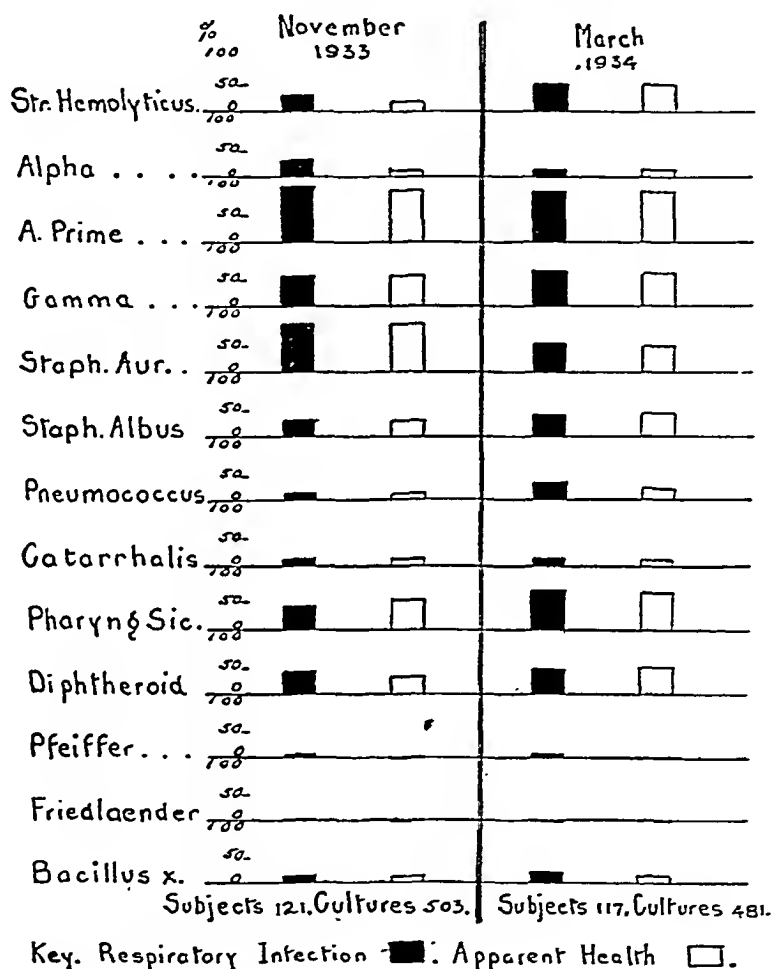


CHART 3. THE PHARYNGEAL FLORA IN RHEUMATIC SUBJECTS DURING RESPIRATORY INFECTIONS AND APPARENT HEALTH.

nificant difference in the frequency or time of appearance of hemolytic streptococci in the throat.

5. There was no noteworthy difference in the incidence of hemolytic streptococci in the throat during apparent health, upper respiratory infection or rheumatic activity.

6. These findings do not suggest any definite relationship between hemolytic streptococci in the throat and rheumatic fever.

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# THE VARIABILITY OF NON-HEMOGLOBIN IRON<sup>1</sup>

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In this paper data on the range of variation of non-hemoglobin iron in human blood will be presented. The significance of these variations in relation to (a) the calculation of hemoglobin, or oxygen capacity from the determinations of total blood iron, and reciprocally, (b) the deduction of values for total blood iron from determinations of hemoglobin or oxygen capacity, will be considered.

Because of the difficulties inherent in the direct determination of hemoglobin there has been a widespread interest in the indirect methods of approach. Of these the oxygen capacity is the most accurate, but there has been an idea in the minds of many that it is a formidable procedure and, as an alternative, there has been a growing tendency to arrive at values for hemoglobin by measurements of total blood iron. Wong (1), Fowweather (2), Sackett (3), Dupray (4), and others have calculated hemoglobin values from determinations of total blood iron. Kennedy (5) believes that total blood iron can be correlated with oxygen capacity and hemoglobin content as determined by colorimetric methods. Murphy, Lynch and Howard (6) have proposed a new index, the "iron index," obtained by dividing the milligrams of iron in 100 cc. of blood by the number of red blood cells in the same volume, as an expression of the concentration of iron in the red blood cells. The validity of these values rests on the assumption that all the iron of the blood is contained within the red blood cells, or combined with hemoglobin, and behaves as a single variable.

In 1898 Abderhalden (7) first showed that there was more iron in the blood of various animals than could be attributed to the hemoglobin alone. He found that the non-hemoglobin iron amounted to 10 per cent of the total blood iron in the ox, 3.6 per cent in the horse, and 6 per cent in the rabbit. Since then Ehrlich and Lazarus (8), Rosin and Jellinek (9), Erben (10), Seiller

(11), Lintzel (12), Freund (13), Barkan (14), Brugsch (15), Warburg and Krebs (16), Langer (17), Locke, Main and Rosbash (18), Dominici (19), McIntosh (20) and many others have verified the presence of non-hemoglobin iron in human blood. The values have varied from minute amounts to 10 per cent of the total blood iron. Barkan (14) showed that there was no relation between the level of hemoglobin and non-hemoglobin iron among twenty-one different individuals nor in the same person under varying conditions. Riecker and Winters (21) found no direct relationship between serum iron and the level of hemoglobin in dogs. Schultze and Elvehjem (22) could obtain no agreement between total blood iron and hemoglobin in fowl.

Because of the fundamental importance of this question to the problems of iron metabolism, iron transport, and the relations between iron and hemoglobin, it seemed desirable to study the magnitude and the variations of non-hemoglobin iron in human beings. With this in mind seventy-two determinations of total blood iron and oxygen capacity were made on the blood of fifty-seven individuals. Patients with a wide variety of disease conditions, as well as normals, were included, in order that the results might give, as far as possible, a picture of the range of both physiologic and pathologic variations. From the work of Barkan (14, 23) who showed that all but a small fraction of the non-hemoglobin iron was contained within the red blood cells, it is obvious that valid figures for non-hemoglobin iron cannot be derived from analyses of blood serum or plasma. Since one gram-molecule of hemoglobin combines with one gram-molecule of iron and one of oxygen, values for hemoglobin-iron can be calculated from measurements of blood oxygen capacity, with the high degree of accuracy inherent in this procedure. To obtain the value for hemoglobin iron the figure for oxygen capacity is multiplied by the factor 2.495. By subtracting this value from the total blood iron determined by analysis, a figure for

<sup>1</sup> Aided by a grant from the research fund of the Yale School of Medicine.

TABLE I  
*Outline of experimental data*

Subject number	Age	Diagnosis	Hemoglobin iron calculated from O <sub>2</sub> capacity	Total iron by analysis	Non-hemoglobin iron	Non-hemoglobin iron
			mgm. per cent	mgm. per cent	mgm. per cent	Total iron
	<i>years</i>					<i>per cent</i>
1.	55	Pernicious anemia, diabetes mellitus	44.8	46.2	1.4	3.0
2.	85	Diabetes mellitus, arteriosclerotic heart disease	51.5	51.7	0.2	0.4
3.	44	Pregnancy, acute nephritis	46.5	48.8	2.3	4.7
4.	69	Cardiovascular disease, dehydration	43.8	45.7	1.9	4.2
5.	63	Diabetes mellitus, arteriosclerosis, generalized	49.0	52.7	3.7	7.0
6.	40	Pernicious anemia	27.4	29.4	2.0	6.8
7.	70	Diabetes mellitus	48.5	51.4	2.9	5.6
8.	65	Paget's disease of bones	27.7	33.7	6.0	17.8
9a.	45	Apoplexy, arteriosclerosis, generalized	44.8	47.8	3.0	6.3
9b.	45	Apoplexy, arteriosclerosis, generalized	45.6	51.0	5.4	10.6
10.	45	Diabetes mellitus, perianal abscess	43.4	45.5	2.1	4.6
11.	48	Cardiac decompensation, severe	40.9	48.1	7.2	15.0
12.	38	Nephritis, subacute; anemia, hypochromic	23.6	25.4	1.8	7.1
13a.	20	Chlorosis	10.8	11.7	0.9	7.7
13b.	20	Chlorosis, iron therapy	29.7	30.4	0.7	2.3
14.	60	Diabetes mellitus, abscess of hand	33.6	36.4	2.8	7.7
15.	16	Acute nephritis	41.9	45.1	3.2	7.1
16.	43	Acute nephritis	35.6	41.0	5.4	13.2
17.	30	Anxiety neurosis, obesity	47.8	53.6	5.8	10.8
18.	73	Diabetes mellitus, arteriosclerosis, generalized anemia, hypochromic	18.8	19.4	0.6	3.1
19.	39	Chronic nephritis with edema, anemia, hypochromic	31.6	34.3	2.7	7.9
20.	57	Leukemia	14.3	14.4	0.1	0.7
21.	60	Arteriosclerosis with heart disease	61.5	62.4	0.9	1.4
22.	17	Hyperthyroidism	52.5	53.1	0.6	1.1
23.	46	Diabetic acidosis	51.0	53.3	2.3	4.3
24a.	56	Polycythemia vera	62.4	64.3	1.9	3.0
24b.	56	Polycythemia vera	62.9	63.2	0.3	0.5
25a.	67	Banti's disease	26.0	26.4	0.4	1.5
25b.	67	Banti's disease	25.4	26.5	1.1	4.2
26.	55	Pernicious anemia, nephritis, acute	34.2	33.0	-1.2	
27.	76	Arteriosclerosis, generalized; anemia, hyperchromic	30.3	29.5	-0.8	
28a.	45	Anemia, hypochromic, due to chronic blood loss	12.6	13.7	1.1	8.0
28b.	45	Anemia, hypochromic, due to chronic blood loss; iron therapy	36.3	36.7	0.4	1.1
29.	43	Cardiovascular renal disease; hypochromic anemia, secondary	34.3	35.4	1.1	3.1
30.	39	Idiopathic hypochromic anemia	28.0	29.2	1.2	4.1
31.	63	Arteriosclerosis, generalized; apoplexy	54.1	55.4	1.3	2.3
32.	63	Polycythemia vera	51.8	53.0	1.2	2.3
33.	39	Idiopathic hypochromic anemia; iron therapy	24.4	25.9	1.5	5.8
34.	44	Idiopathic hypochromic anemia	25.1	28.7	3.6	12.5
35a.	41	Bleeding peptic ulcer	21.5	23.1	1.6	6.9
35b.	41	Bleeding peptic ulcer	38.3	39.1	0.8	2.0
36a.	44	Pernicious anemia	17.2	18.7	1.5	8.0
36b.	44	Pernicious anemia, liver therapy	26.0	30.5	4.5	14.8
37a.	58	Emphysema, polycythemia	59.0	60.7	1.7	2.8
37b.	58	Polycythemia, phenylhydrazine therapy	50.4	51.2	0.8	1.6
37c.	58	Polycythemia, phenylhydrazine continued	43.5	43.8	0.3	6.9
38a.	60	Polycythemia vera	70.7	71.5	0.8	1.1
38b.	60	Polycythemia vera, phenylhydrazine therapy	56.3	63.6	7.3	11.5
39.	30	Normal	51.0	52.9	1.9	3.6
40a.	28	Normal	49.6	52.0	2.4	4.6
40b.	28	Normal	52.2	56.0	3.8	6.7
41.	30	Normal	46.6	47.3	0.7	1.5
42a.	30	Normal	44.2	49.7	5.5	11.1
42b.	30	Normal	49.2	54.3	5.1	9.4

TABLE I (continued)

Subject number	Age	Diagnosis	Hemoglobin iron calculated from O <sub>2</sub> capacity	Total iron by analysis	Non-hemoglobin iron	Non-hemoglobin iron
						Total iron
	years		mgm. per cent	mgm. per cent	mgm. per cent	per cent
42c.	30	Normal	47.8	50.4	2.6	5.2
43a.	31	Normal	50.2	52.7	2.5	4.7
43b.	31	Normal	47.3	49.2	1.9	3.9
44.	35	Normal	48.3	50.7	2.4	4.7
45a.	25	Normal	45.4	48.5	3.1	6.4
45b.	25	Normal	49.1	49.1	0.0	0.0
46.	21	Normal	44.2	46.8	2.6	5.6
47.	25	Normal	54.7	58.6	3.9	6.7
48.	25	Normal	51.1	54.7	3.6	6.6
49.	26	Normal	48.8	51.4	2.6	5.1
50.	26	Normal	47.2	48.3	1.1	2.3
51.	21	Normal	52.8	57.0	4.2	7.4
52.	38	Normal	57.4	60.2	2.8	4.7
53.	26	Normal	45.7	46.3	0.6	1.3
54.	21	Normal	42.2	43.3	1.1	2.5
55.	22	Normal	45.1	47.1	2.0	4.2
56.	28	Normal	45.2	45.9	0.7	1.5
*57.	25	Normal	54.2	69.5	15.3	22.0

Mean non-hemoglobin iron	2.2 mgm. per cent	Standard deviation $\pm 1.74$ mgm. per cent
$\frac{\text{Non-hemoglobin iron}}{\text{Total iron}}$	5.3 per cent	Standard deviation $\pm 3.9$ per cent
Mean error of duplicate determinations of total iron	0.38 mgm. per cent	Standard deviation $\pm 0.46$ mgm. per cent
Mean error of duplicate determinations of oxygen capacity in terms of hemoglobin iron	0.38 mgm. per cent	Standard deviation $\pm 0.31$ mgm. per cent

\*Omitted from statistical consideration.

true non-hemoglobin iron can be obtained. Barkan (24) attempted to arrive at values for non-hemoglobin iron by ultrafiltration of hemolyzed blood, and Winegarden and Borsook (25) by dialysis; but in view of the unstable nature of hemoglobin when subjected to manipulation, these methods cannot be considered reliable.

Oxygen capacity was determined in duplicate by the carbon monoxide method of Van Slyke and Hiller as described by Peters and Van Slyke (26). Total blood iron was measured in duplicate or triplicate by volumetric titration with titanium according to the technique recently described (27). Data concerning the sensitivity of this method as well as recovery experiments are given in that paper.

#### RESULTS

The experimental findings are presented in Table I.

The value for non-hemoglobin iron of Case 57 was omitted in the mathematical treatment of results because it lies so far beyond the range of the other findings that it is highly probable that some gross technical error was undetected.

In this series an average of 5.3 per cent of the total iron was found uncombined with active hemoglobin. This is in close agreement with the work of Barkan (24) who reports that the free iron of the blood amounts to from 5 to 6 per cent of the hemoglobin iron. In Figure 1 the values for non-hemoglobin iron are plotted against oxygen capacities. It can be readily seen that there is no correlation between non-hemoglobin iron and oxygen capacity. These results indicate that attempts to arrive at values for hemoglobin by determination of total blood iron or vice versa, are subject to an average error of 5.3 per cent with a standard deviation of 3.9 per

TABLE I  
Outline of experimental data

Subject number	Age	Diagnosis	Hemoglobin iron calculated from O <sub>2</sub> capacity	Total iron by analysis	Non-hemoglobin iron	Non-hemoglobin iron
						Total iron
	<i>years</i>		<i>mgm. per cent</i>	<i>mgm. per cent</i>	<i>mgm. per cent</i>	<i>per cent</i>
1.	55	Pernicious anemia, diabetes mellitus	44.8	46.2	1.4	3.0
2.	85	Diabetes mellitus, arteriosclerotic heart disease	51.5	51.7	0.2	0.4
3.	44	Pregnancy, acute nephritis	46.5	48.8	2.3	4.7
4.	69	Cardiovascular disease, dehydration	43.8	45.7	1.9	4.2
5.	63	Diabetes mellitus, arteriosclerosis, generalized	49.0	52.7	3.7	7.0
6.	40	Pernicious anemia	27.4	29.4	2.0	6.8
7.	70	Diabetes mellitus	48.5	51.4	2.9	5.6
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16.	43	Acute nephritis	35.6	41.0	5.4	13.2
17.	30	Anxiety neurosis, obesity	47.8	53.6	5.8	10.8
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45a.	25	Normal	45.4	48.5	3.1	6.4
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*57.	25	Normal	54.2	69.5	15.3	22.0

Mean non-hemoglobin iron	2.2 mgm. per cent	Standard deviation $\pm$ 1.74 mgm. per cent
Non-hemoglobin iron Total iron	5.3 per cent	Standard deviation $\pm$ 3.9 per cent
Mean error of duplicate determinations of total iron	0.38 mgm. per cent	Standard deviation $\pm$ 0.46 mgm. per cent
Mean error of duplicate determinations of oxygen capacity in terms of hemoglobin iron	0.38 mgm. per cent	Standard deviation $\pm$ 0.31 mgm. per cent

\*Omitted from statistical consideration.

true non-hemoglobin iron can be obtained. Barkan (24) attempted to arrive at values for non-hemoglobin iron by ultrafiltration of hemolyzed blood, and Winegarden and Borsook (25) by dialysis; but in view of the unstable nature of hemoglobin when subjected to manipulation, these methods cannot be considered reliable.

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cent, and a possible error as great as 17.8 per cent. The scatter of the actual values of non-hemoglobin iron is given in Figure 2. The wide range of variations of non-hemoglobin iron is apparent.

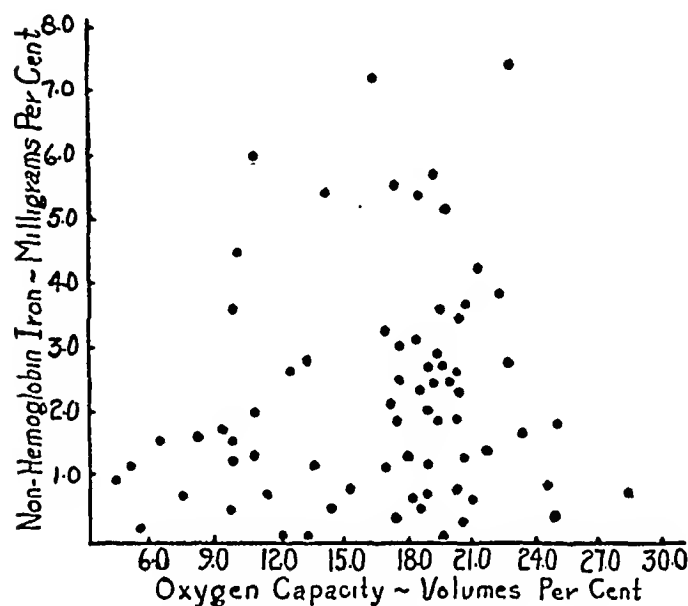


FIG. 1. CORRELATION CHART.

The relation between non-hemoglobin iron and oxygen capacity.

In agreement with Barkan (23), it was evident from simultaneous determinations of total blood iron, oxygen capacity, and serum iron, that the serum contained only a fraction of the non-hemoglobin iron. This will be considered in detail in another report.

#### SUMMARY

From determinations of oxygen capacity and total blood iron in fifty-seven subjects under various conditions, values for non-hemoglobin iron were obtained. It was found that non-hemo-

globin iron varies widely and is a signification of total iron. For these reasons, a correlate values for hemoglobin and oxygen capacity with determinations of total blood fruitless.

The generous assistance of Dr. Anna J. [Name] man is appreciatively acknowledged.

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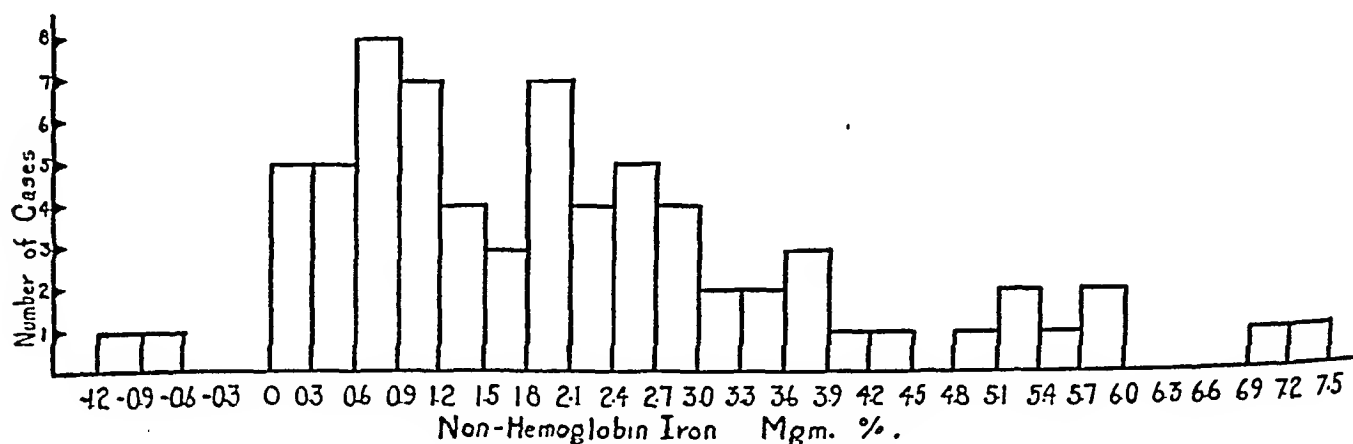


FIG. 2. THE DISTRIBUTION OF VALUES FOR NON-HEMOGLOBIN IRON.

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# THE EFFECT OF PROTEIN ON THE CARBOHYDRATE TOLERANCE OF TWO CASES HAVING COMBINED DIABETES MELLITUS AND PERNICIOUS ANEMIA<sup>1</sup>

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We have studied two subjects having both diabetes mellitus and pernicious anemia during a six and nine months' period of hospitalization. This combination of diseases has been recently reviewed by Root (1).

Marked fluctuations in carbohydrate tolerance were observed to follow changes in protein content of the diet. It is well known that the composition of the diet of the normal (2) as well as of the diabetic patient plays an important part in the carbohydrate tolerance. It is generally recognized (3) that the adult diabetic does best on a diet containing 0.66 to 1.0 gram of protein per kilogram of body weight per diem. The use of limited amounts of protein in the diets is so universal that the reason for its curtailment is often forgotten. Consequently, in the therapy of diseases complicating diabetes the tendency has been to give the diet prescribed for the complicating disease and if glycosuria occurs to control it with insulin. If this complicating disease is one in which a high protein diet is advisable, such as pernicious anemia, a real danger exists. The carbohydrate tolerance of these subjects, already impaired, may be seriously damaged.

Prior to the method of treatment by undernutrition, diabetic patients were placed on a diet consisting entirely of protein and fat. As Joslin (3) expresses it, these patients were "urged to devour, not merely eat, protein and fat ad libitum." In consequence, there were no mild diabetics among those so treated. The mildly diabetic, due to lack of carbohydrate in the diet and the excess of protein and fat, were made moderately severe. The moderately severe became severe, while the latter died in ketosis or barely escaped with their lives. During the era of undernutrition, patients were maintained aglycosuric whose

urine had never before been consistently sugar free, and the average duration of life of the diabetic increased. The success of this treatment must be credited to undernutrition and the sharp curtailment of protein in the diet. Falta and Gigon (4) in 1907 noted that an increase in protein of the diet of a severe diabetic caused more glycosuria than an increase in carbohydrate. Klemperer (5) showed that even dextrose could be assimilated to a considerable degree by a severe diabetic patient provided the protein in the diet was low.

The rapidity with which the effect of an excess of protein when given with a low carbohydrate, high fat diet manifests itself was demonstrated by a case reported by Gephart, Aub, DuBois, and Lusk (6). This patient's urine was sugar free on a diet of C. 40 to 50 grams, P. 94 grams and F. about 100 grams. He complained so bitterly that the diet was changed to C. 3 grams, P. 156 grams and F. 230 grams, giving double the calories and approximately the same carbohydrate equivalent. On the second day of this diet the D:N ratio was 3.48. The deleterious effect of this diet may have been caused by increase in protein, an increase in fat, or by an increase in total calories. Evidence that the deleterious effect of this type of diet was due chiefly to the excess protein was presented by Geyelin and DuBois (7). In their patient, on a diet of C. 51 grams, P. 54 grams, F. 56 grams, the D:N ratio averaged 1.81. A single day on a diet of C. 23.5 grams, P. 118.6 grams, F. 41 grams, which was isocaloric with the preceding diet, and contained 10 grams more available carbohydrate, resulted in a D:N ratio of 3.97. The following day the diet was rearranged to C. 0.4 gram, P. 99 grams, F. 5.6 grams, almost halving the calories and reducing the available carbohydrate to below 10% of the original diet; the D:N ratio became 1.01. This case demonstrates that the increase in carbohydrate tolerance was not due to increase in total calories.

<sup>1</sup> Presented in abstract before the Interurban Clinical Club, New York, December, 1934.

fat, or in available carbohydrate. It demonstrates that the moderately severe diabetic may be converted into a severe diabetic by an increase in protein. Confirmation of the damaging effect of excess protein may be read in the work of Wilder, Boothby, and Beeler (8). Lusk (9) calls it "as clear-cut a piece of metabolism artistry as may well be conceived." These authors showed that the sugar tolerance is depressed by an increase in total calories, but more markedly depressed by an increase in protein than by isocaloric amounts of fat. They further state that the protein effect is not due to the sugar or ketogenic bodies derived from the protein but to "some other more specific action of protein, the result of which is to interfere with the mechanism of sugar utilization."

Recently Schloss (10) has presented evidence which indicates that high protein diets may not necessarily be harmful in some individuals. However, results presented in a second paper (11) indicate that glycosuria was greater on a high protein than on a high fat diet, whether preceded or followed by the latter.

Von Noorden (12), Falta and Gigon (4), and Joslin (3) emphasized the danger of excess protein in the diet in diabetes mellitus. Woodyatt (13) demonstrated the necessity for keeping diabetics on a diet containing enough calories to prevent consumption of body protein. He emphasized that when the patient is forced to fall back on his own tissues for food he can draw only upon the materials that are there; if fat is not present the protein burns. Excessive consumption of body protein may explain some of the failures of treatment by the method of undernutrition.

Newburgh and Marsh (14) effectively demonstrated this point in a group of 43 diabetics. Their diet consisted of C. 10 to 30 grams, P. 10 to 40 grams, and F. 20 to 175 grams. The upper margin for fat in grams when placed at twice the carbohydrate plus one-half the protein was well exceeded in all their diets. They demonstrated that a diabetic subject may be expected to become and remain sugar free without acidosis on a high fat diet containing sufficient calories to prevent undernutrition provided the protein was low.

From the available literature on the effect of

protein in the diabetic's diet it has been demonstrated:

1. That pure protein-fat diet is extremely deleterious.
2. On low carbohydrate diets an increase in protein as a rule increases the severity of the diabetes out of all proportion to the extra sugar derived from protein.
3. Isocaloric increases in protein are generally more harmful than isocaloric increases in fat.
4. That acidosis does not occur when the ketogenic-anti-ketogenic ratio is exceeded provided the protein is minimal.

Will the high carbohydrate diet protect, delay, or minimize the deleterious action of an excess protein in the diet of the diabetic? Will the deleterious effects of excess protein be manifest if long continued or severe glycosuria be prevented? Will the impaired tolerance be permanent? We believe the study of our two cases indicates the answer to these questions.

#### CASE REPORTS

##### *Case I*

M. K., a 59 year old native white widow, was admitted to the medical wards of the Bellevue Psychiatric Division on September 28, 1933. She was admitted to another hospital earlier the same day, but showed mental symptoms and was immediately transferred to this hospital. On admission no history was obtained; she was semi-comatose, with a heavy odor of acetone on the breath and evidence of dehydration and emaciation. The breathing was of the Kussmaul type. Urine contained much acetone and diacetic acid and 3 per cent sugar. The blood sugar was 375 mgm. per cent, and nonprotein nitrogen 39 mgm. per cent. Treatment for diabetic ketosis was immediately instituted with the result that the patient was alert and cooperative in four hours and the urine free from acetone in seven hours. Further examination revealed a marked pallor, smooth tongue and signs of well advanced combined system disease of the spinal cord. The blood count was 1.9 million red cells, hemoglobin 6.4 grams per 100 cc. Study of the stained smear revealed marked oval macrocytosis. The total leukocyte count was 3,100, with a differential formula of polymorphonuclear leukocytes 77, lymphocytes 23, monocytes 0. Gastric analysis, using the alcohol test meal and followed by histamine, showed absence of free hydrochloric acid and extremely low total acidity. Stool examinations were negative for ova, parasites or blood. Roentgen studies of the gastro-intestinal tract were negative.

Following recovery from the ketosis the following

pertinent history was obtained: In 1928 the patient had developed symptoms of weakness and paresthesias, for which she had entered another hospital. While there she was considered to have Addisonian pernicious anemia, and a remission was induced by administration of liver extract. Following discharge she continued to take a diet rich in green vegetables and meats, with liver twice a week. She remained in good health until June, 1933, when she lost her appetite and stopped eating liver and meat. A month later she noted return of weakness and paresthesias and loss of weight. Polyuria and polydipsia began two weeks prior to admission, and one week before admission she was told she had diabetes. At this time the patient was living in a home for the aged, and no treatment was instituted.

given a diet of C. 150 grams, P. 70 grams, F. 85 grams, and 13 days later the carbohydrate was increased to 200 grams. On this diet and 30 to 36 units of insulin daily in divided doses the urine became free from sugar within one week. The insulin was then progressively decreased to a single morning dose of 10 units. It should be noted that the insulin requirement fell in spite of a moderate fever of unknown origin and an increase in carbohydrate from 150 to 200 grams. Attempts to decrease the insulin to 5 units on October 25 and October 26 resulted in the appearance of 9 grams of sugar in the urine on each of these two days. During the last two weeks of this period the patient gained only 0.7 gram of hemoglobin and less than 400,000 red cells. Because of the inadequate hematological response we determined to re-

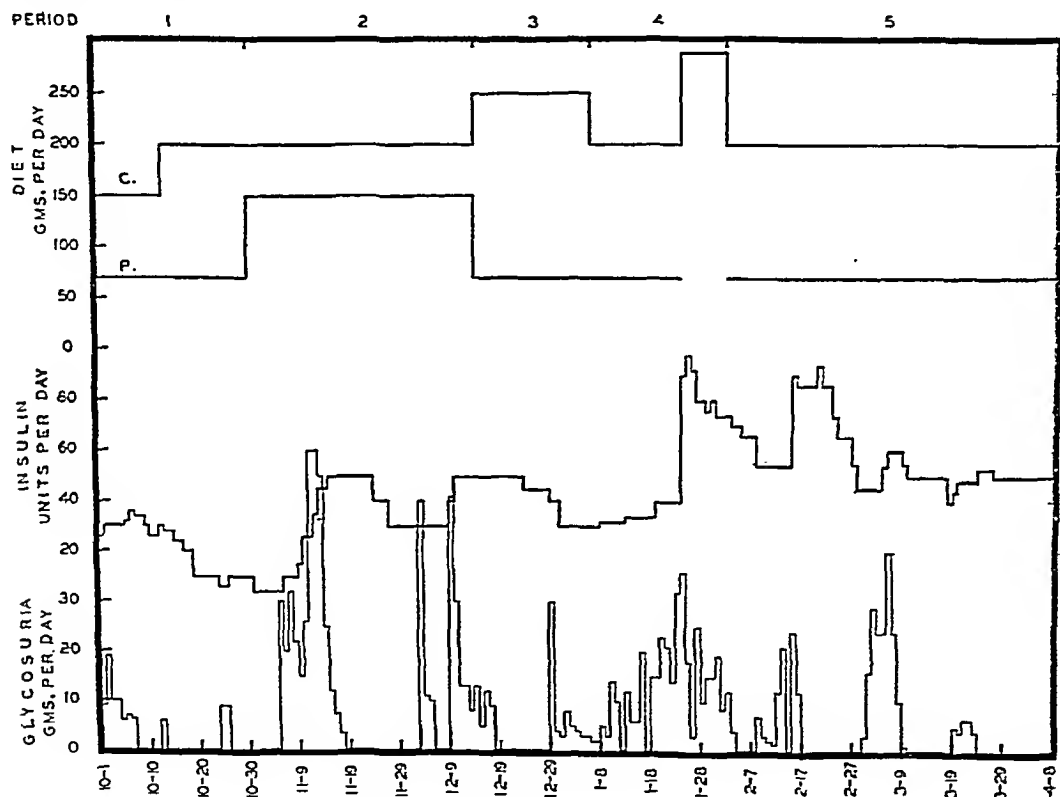


FIG. 1. OBSERVATIONS ON CASE I, M. K.

Liver therapy was instituted the day of admission, the patient receiving 3 cc. of Lederle's liver extract for parenteral use, containing the material derived from 100 grams of liver, on September 28, 29, and 30. An increase in reticulocytes followed, reaching a maximum of 11 per cent on October 3. From this point on the course of events can best be followed in stages, and by reference to Figure 1.

*Period 1.* During this 30 day period the patient received 1 gram of iron daily in the form of ferric ammonium citrate, and two injections each week of 3 cc. Lederle's liver extract for parenteral use. She was

arrange her diet more in accordance with that given patients with uncomplicated anemia. This plan was carried out in Period 2.

*Period 2.* The diet given contained C. 200 grams, P. 155 grams, F. 85 grams, and parenteral liver and iron by mouth was continued as in Period 1. During this period of 45 days a satisfactory hematological response was observed. The red cell count reached five million, and the hemoglobin 12 grams per 100 cc. on December 6, 43 days following institution of the high protein diet. On the second day of this period, in spite of an increase of 49 grams in available carbohydrate, a mild reaction to

fat, or in available carbohydrate. It demonstrates that the moderately severe diabetic may be converted into a severe diabetic by an increase in protein. Confirmation of the damaging effect of excess protein may be read in the work of Wilder, Boothby, and Beeler (8). Lusk (9) calls it "as clear-cut a piece of metabolism artistry as may well be conceived." These authors showed that the sugar tolerance is depressed by an increase in total calories, but more markedly depressed by an increase in protein than by isocaloric amounts of fat. They further state that the protein effect is not due to the sugar or ketogenic bodies derived from the protein but to "some other more specific action of protein, the result of which is to interfere with the mechanism of sugar utilization."

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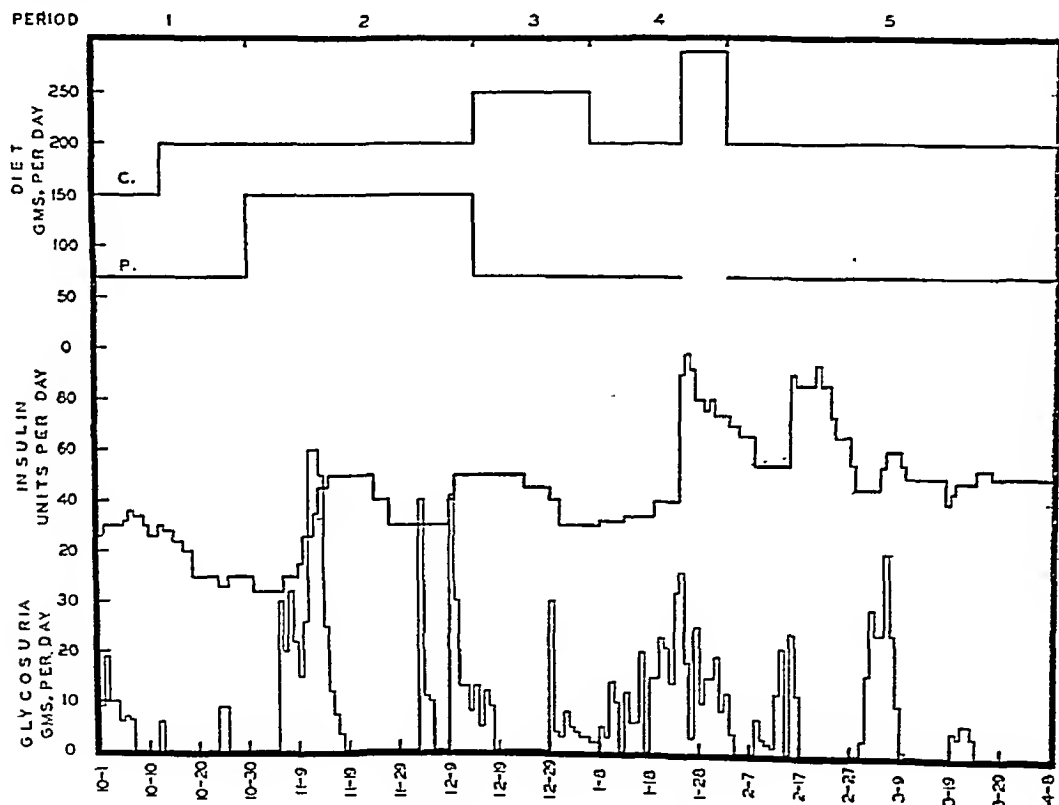


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*Period 1.* During this 30 day period the patient received 1 gram of iron daily in the form of ferric ammonium citrate, and two injections each week of 3 cc. Lederle's liver extract for parenteral use. She was

arrange her diet more in accordance with that given patients with uncomplicated anemia. This plan was carried out in Period 2.

*Period 2.* The diet given contained C. 200 grams, P. 155 grams, F. 85 grams, and parenteral liver and iron by mouth was continued as in Period 1. During this period of 45 days a satisfactory hematological response was observed. The red cell count reached five million, and the hemoglobin 12 grams per 100 cc. on December 6, 43 days following institution of the high protein diet. On the second day of this period, in spite of an increase of 49 grams in available carbohydrate, a mild reaction to

insulin occurred. The dose of insulin was then reduced to 5 units daily. Five days later 30 grams of sugar appeared in the urine, and during the next 15 days, in spite of constantly increasing amounts of insulin, moderate glycosuria continued. To make the urine sugar free 50 units of insulin were necessary. This dosage of insulin was continued for six days in which no glycosuria or insulin reactions occurred. On November 24 the dose was reduced to 40 units daily, and on November 27 to 30 units daily. On December 3 about 35 grams of sugar was found in the urine, and about 10 grams on the next two succeeding days. Glycosuria ceased for three days, and then recurred, necessitating an increase in insulin to 40 units on December 9 and to 50 units on December 10. This amount of insulin failed to prevent glycosuria, about 10 grams of sugar having been found in the urine on December 14. We believed that this increase in severity of the diabetes could be attributed to the excess protein, and consequently the diet was changed to that in Period 3.

*Period 3.* The diet given contained C. 250 grams, P. 70 grams, F. 85 grams. This diet during a period of 23 days had the same carbohydrate equivalent per diem as the preceding high protein, and furnished 140 less total calories. The dose of 50 units of insulin was continued, and within four days glycosuria was absent. Insulin was gradually decreased to 30 units daily. On this diet and dosage of insulin, about 5 grams of sugar per diem appeared in the urine for the last five days of this period. We believe it probable that the insulin requirement would have decreased progressively had not the occurrence of fever ended this period.

*Period 4.* This period of infection lasted for 25 days. On January 8 a rhinitis developed, and although the patient was confined to bed a temperature of 101° developed on January 11. No definite cause of the low grade fever could be determined. On January 24 a typical facial erysipelas developed, with fever of 105°. The diet was immediately changed to one consisting of almost pure carbohydrate. She received food every two hours day and night with a dosage of insulin depending on urinary findings taken prior to each feeding. The patient went through this severe infection without acidosis. The red cells fell to three million, in spite of transfusions of 500 cc. of whole blood on January 25, 26, and 27. On January 28 the erysipelas was fading, the insulin requirement diminishing, and the temperature subsiding, and by February 2 the patient was free of fever, at which time the diet of Period 5 was instituted.

*Period 5.* This period of readjustment of diet and insulin following infection continued over the next 60 days till discharge of the patient. She was given a diet containing C. 200 grams, P. 70 grams, F. 85 grams. On this diet 90 units of insulin per diem, in divided doses, were required at first to control glycosuria. We were able gradually to reduce the dosage of insulin, but at no time could it be decreased below 50 units daily without the appearance of glycosuria, as shown in Figure 1, between February 27 and March 9, and March 19 to March

24. We lost track of this patient following discharge and are therefore unable to make a report of the course of events subsequently.

## Case II

R. G., an unmarried Russian Jewess, 28 years old, was admitted for mental observation to the medical wards of the Bellevue Psychiatric Division on August 30, 1933. Examination showed a poorly nourished woman, appearing chronically ill, bedridden and with a moderate pallor. There was a smooth tongue, marked weakness of both lower extremities with absent tendon reflexes, absent postural and vibratory sense, and an extensor plantar response. The heart and lungs were normal. No viscera were palpable. Examination of the blood showed 8.8 grams hemoglobin per 100 cc. and 2.58 million red blood cells per cu. mm. The color index was 1.0, saturation index 0.80, and the volume index 1.26. The smear showed marked poikilocytosis and anisocytosis, and a number of oval macrocytes. The white blood cells numbered 5,800, with a differential count as follows: polymorphonuclear leukocytes 70, lymphocytes 28, and monocytes 2. The serum bilirubin was 0.74 mgm. per cent, and gave a delayed direct Van den Bergh reaction. Using the alcohol test meal, followed by histamine, no free hydrochloric acid could be demonstrated in the gastric contents. A postabsorptive blood sugar was 100 mgm. per cent. The urine was normal. Repeated stool examinations were negative for blood or parasites. Complete roentgenological study of the gastro-intestinal tract revealed it to be normal. It seemed probable that this patient had Addisonian pernicious anemia, but to obtain more evidence on this point the patient was treated by parenteral administration of a preparation of yeast; no reticulocyte response followed. On September 12 parenteral liver therapy was started; 3 cc. of Lederle's parenteral liver extract, containing the material derived from 100 grams of whole liver were administered for three successive days. An adequate reticulocyte response followed this procedure. Simultaneously with the reticulocyte response a marked change occurred in the patient. The delusions of identity disappeared; she became agreeable and co-operative, and ate the diet prescribed. Before the increase of reticulocytes the patient had to be fed through a tube. She had insisted volubly that she was the Czarina of Russia and had been kidnaped. Subsequently an adequate history was obtained, both from the patient and her family. The family history failed to reveal nervous or mental disease, anemia, or diabetes. Except that she had had the common childhood diseases the patient had been well till July, 1932, when she noted increasing weakness and fatigue, and difficulty in walking, accompanied by a loss of 25 pounds in weight. She had entered another hospital where a diagnosis of a microcytic hypochromic type of anemia was made. Mildly abnormal neurological signs were reported to have been present at that time. The postabsorptive blood sugar was 98 mgm. per cent, and the urine normal. She was treated with liver and iron and made a fair recovery, remaining well for a year. About the first of August,

1933, the patient developed mental symptoms, weakness, fatigue, and a progressive inability to walk. After about a month the family, because they were unable to persuade the patient to eat, and because of her delusions of identity, brought her to the hospital for mental observation.

marked tenderness was elicited in the right costo-vertebral triangle. The pyelitis rapidly cleared by the usual expectant treatment, and the temperature was normal by October 25. There was a mild recurrence of fever from December 6 to 8, after which the patient was free of fever.

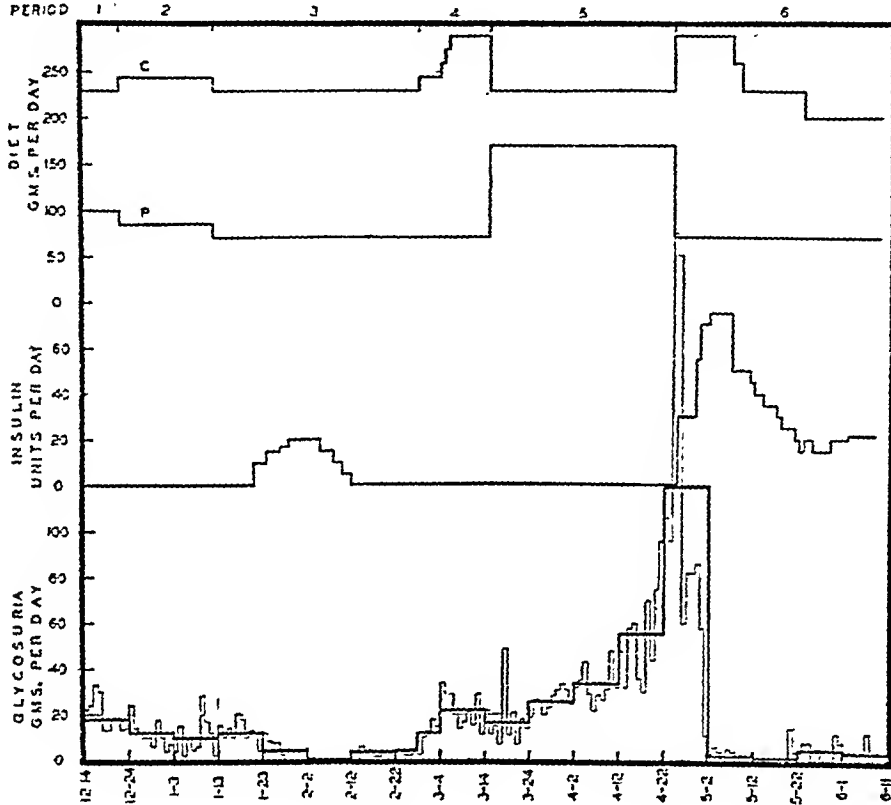


FIG. 2. OBSERVATIONS ON CASE II, R. G.

Following the rise of reticulocytes the patient was given a diet which was rich in protein and green vegetables, in addition to one-half pound of liver daily. Two parenteral injections of liver, each containing the material derived from 100 grams of liver, were given weekly, as well as 1 gram daily of iron by mouth. A satisfactory level of hemoglobin and red cells was reached by December 1, but on account of the marked neurological changes confining the patient to bed or a wheel chair hospitalization was continued. Parenteral administration of liver and iron by mouth was continued, as well as physiotherapy. One-half pound of liver daily was added to the high caloric, high protein diet for anemia.

On admission, August 30, 1933, the patient had a temperature of  $101^{\circ}$ . The fever continued to run a low grade course till September 10, after which the temperature became normal. No adequate explanation can be offered for this period of fever. On October 15 the temperature suddenly rose to  $102^{\circ}$ . On the next day clumps of pus cells were discovered in the urine and

Casual urine specimens, nine in number, were negative for sugar till November 27, at which time a trace appeared; a morning specimen of urine on November 29 contained 0.5 per cent sugar; glycosuria was absent till December 8, when it recurred. On December 11 the fasting blood sugar was 131 mgm. per cent, rising to 172 mgm. two hours after breakfast. On December 14 the blood sugar was 175 mgm. per cent fasting, and 230 and 207 mgm. per cent two and three and one-half hours after breakfast respectively. The course of events with this patient can now best be followed in periods, and by reference to Figure 2.

*Period 1.* During this period of 8 days the diet contained C. 230 grams, P. 100 grams, F. 85 grams, and no insulin was given. This diet contained about one-half the amount of protein given in the unweighed anemia diet. The diabetes during this period was mild, as shown by the average daily excretion of 18 grams of urinary sugar, 278 grams of the 296 grams of carbohydrate available in the diet having been utilized.

*Period 2.* The diet during this period of 21 days was



maintained isocaloric with that in Period 1 by decreasing the protein 15 grams and increasing the carbohydrate by 15 grams, making the dietary formula C. 245 grams, P. 85 grams, F. 85 grams. The carbohydrate equivalent was 6 grams more than that in Period 1. The amount of sugar in the urine decreased from an average of 18 grams to 13 grams for the first 10 days on this diet, and to 9 grams during the second 10 day period. This indicates the utilization of 289 and 293 of the 302 grams of available carbohydrate in the two consecutive 10 day periods, as compared with 278 grams dextrose available in Period 1.

*Period 3.* The patient in Period 2 had demonstrated the ability to utilize 289 grams of dextrose with a protein intake of 85 grams per diem. During the third period, of 46 days, the carbohydrate equivalent was reduced to 279 grams by reducing the carbohydrate and protein in the diet each by 15 grams, making the dietary formula C. 230 grams, P. 70 grams, F. 85 grams. The glycosuria continued at about 10 grams per diem. On January 20, insulin was first given, 20 units being required to prevent glycosuria. We were then able to progressively reduce the insulin to 5 units daily without the recurrence of glycosuria. When administration of insulin was omitted entirely the average amount of sugar excreted daily was 2.5 grams during the last 15 days of this period. This degree of glycosuria was practically constant and showed no tendency to increase. This last period of 15 days seemed to us an adequate control period for an acute experiment, for we recognized that any change to be significant must be marked and definite.

*Period 4.* During this 16 day period the diet was changed to C. 290 grams, P. 70 grams, F. 85 grams. This increase of 60 grams in carbohydrate was accomplished over an eight day period. The addition of 60 grams of carbohydrate resulted in a daily average of 22 grams of sugar over a 10 day period. Here again there was no tendency for the glycosuria to increase over the last 10 days of this period.

*Period 5.* This period of high protein feeding lasted for 41 days. The extra 60 grams of carbohydrate added in Period 4 was omitted, and 100 grams of protein added, making the dietary formula C. 230 grams, P. 170 grams, F. 85 grams. The available carbohydrate of this diet was almost identical with that in Period 4 and was, for practical purposes, isocaloric. During the first 10 day period of this diet the average daily sugar excretion decreased to 17 grams from the daily average excretion of 22 grams for the last 10 days of Period 4. In the second 10 day period, from May 24 to April 2, the average daily excretion was 25 grams of sugar, and from this point on the severity of the diabetes progressed rapidly: The average from April 2 to April 12 was 33 grams; from April 12 to April 22, 55 grams. On April 22 and 23, 120 grams of sugar was found in the urine, and on April 24, 275 grams, accompanied by thirst and polyuria but no acetone bodies. The patient on this day utilized only 62 of the 337 grams of available carbo-

hydrate in the diet. The patient was now returned to the diet in Period 4.

*Period 6.* The extra 100 grams of protein added to the diet in Period 5 was replaced by 60 grams of carbohydrate, making the dietary formula C. 290 grams, P. 70 grams, F. 85 grams. With this diet 75 units of insulin daily in four doses was required to reduce the glycosuria to an average of 5 grams daily. With the same diet in Period 4, without insulin, the patient excreted an average of 22 grams of sugar daily. Exogenous insulin probably accounted for the utilization of 150 grams of sugar, and with 5 grams appearing in the urine it may be calculated that this patient utilized over this five day period (May 2 to May 7) 184 of the 339 grams of available carbohydrate in the diet. The carbohydrate in the diet was then cut to 260 grams and, two days later, to 230 grams. Insulin dosage was gradually reduced to 25 units daily. At this point, 25 days after termination of the high protein diet, it may be calculated that the patient was utilizing 229 grams of the 279 grams of available carbohydrate in her diet. Attempts to reduce insulin below 25 units daily resulted in glycosuria. On the same diet without insulin in Period 4 an average of 2.5 grams of sugar appeared in the daily urine. On May 23, preparatory to discharge from the hospital, the carbohydrate in the diet was reduced to 200 grams. On this diet the insulin dosage was 22 units, with appearance of an average of 6 grams of urinary sugar from June 2 to discharge to a hospital for chronic diseases on June 11. The patient six months later was on a diet of C. 90 grams, P. 75 grams, F. 90 grams, and taking 30 units of insulin without glycosuria.

#### COMMENT

The addition of an excess of protein to the diet of these two individuals having pernicious anemia did not result in an immediate increase in severity of the diabetes. In M. K., following the addition of 80 grams of protein to her diet, a mild insulin reaction occurred on the second day, causing us to decrease the dose of insulin from 10 to 5 units daily. In R. G., the addition of 100 grams of extra protein and a simultaneous decrease in the carbohydrate of the diet by 60 grams was followed by a diminution in the daily excretion of urinary sugar for 10 days. The addition of excess protein to the diet in the cases reported by Gephart, Aub, DuBois and Lusk (6), Geyelin and DuBois (7), and by Wilder, Boothby and Beeler (8) resulted in the immediate increase in the severity of the disease. We believe it possible that the high carbohydrate content of the diet may have protected our patients from the immediate deleterious effects of the added protein. The carbohydrate content of the diets used by the

previous observers was exceedingly low. In two instances only 3 grams or less of carbohydrate was allowed daily, and in the other 23.5 grams daily was permitted. In our cases, M. K. was allowed 200 grams, and R. G. 230 grams of carbohydrate at the time they were receiving the extra protein.

The ultimate result in our two cases of an excess of protein in the diet was deleterious. The influence of adding protein without otherwise changing the diet is effectively demonstrated in Periods 1, 2, and 3 in M. K. This patient demonstrated the ability to remain free of glycosuria when 10 units of insulin were given daily (Period 1) with a dietary formula of C. 200 grams, P. 70 grams, F. 85 grams. The addition of 80 grams of protein was followed by glycosuria on the fifth day, and in spite of progressively increasing the dose of insulin, glycosuria persisted for 15 days. The progressive nature of the impairment may be noted in that from November 19 to 25 the urine was sugar free when 50 units of insulin were given daily, but on the same amount of insulin, glycosuria was present from December 10 to 14. This also shows that the deleterious effect of an excess of protein was manifest in the absence of glycosuria. The 80 grams excess of protein, continued for 45 days, was followed by an increase of 40 units of insulin daily, which amount was not quite sufficient, at the end of the period, to prevent glycosuria. The extra 49 grams of carbohydrate made available by the 80 grams of protein required an increase of 40 units of insulin. On cutting the extra 80 grams of protein and substituting an equivalent amount of carbohydrate (Period 3) and maintaining the same amount of insulin, glycosuria was absent by the fifth day. Likewise it is to be noted that slight glycosuria does not preclude improvement in carbohydrate utilization. A progressive improvement occurred, so that by January 1, 17 days after omitting the excess protein, the insulin requirement was 30 units, with only 2 to 8 grams of sugar in the daily 24 hour urine. We believe an increase in insulin of 3 to 5 units would have made the urine sugar free at this stage. We believe this patient would have continued to improve had not Period 3 been interrupted by a severe infection. Our data on this patient from

this time can not be used to study the effect of protein.

The deleterious effect of an excess of protein was not minimized by a reduction in the carbohydrate of the diet equivalent to the available dextrose in the protein. This is demonstrated in the case of R. G. (Periods 4, 5, and 6). In Period 3 the patient demonstrated an ability to utilize a diet of C. 230 grams, P. 70 grams, F. 85 grams without insulin with an average of only 3 grams of sugar in the urine over a 15 day period (February 12 to 27). In Period 4 the carbohydrate over a week's time was increased by 60 grams to 290 grams with an average excretion over a 10 day period (April 4 to 14) of 22 grams of sugar. There was no indication during this period that the glycosuria was increasing. The 60 grams of extra carbohydrate was replaced by 100 grams of protein containing the same amount of available carbohydrate. Following the first 10 day period in which the average sugar excretion slightly decreased, there was a progressive and constantly increasing glycosuria. Between the 30th and 40th day the average daily glycosuria amounted to 75 grams, indicating that the patient was now utilizing 262 of the 337 grams of available carbohydrate in her diet. At this point the daily urine volume suddenly increased (April 22) to be followed by 140 grams of urinary sugar on April 22, and 275 grams on April 23. Due to the marked glycosuria and diuresis, with loss of serum electrolytes in the urine, we felt that it was no longer safe to continue this diet. Consequently, in Period 6, the 100 grams of protein was replaced by 60 grams of carbohydrate, and insulin given. At this time, May 6, the patient required 75 units of insulin to prevent glycosuria. Probably this exogenous insulin utilized 150 grams of carbohydrate, indicating that this patient was utilizing 189 grams of the 339 grams of available carbohydrate in her diet. Within 13 days, therefore, after discontinuing the high protein diet the carbohydrate tolerance of the patient had increased from 112 to 189 grams. During the next 10 days the improvement was more rapid, so that 25 days after discontinuing the excess protein the patient was utilizing 229 of the 279 grams of available carbohydrate in her diet. This is the last available accurate calculation of tolerance, for when we reduced her diet

maintained isocaloric with that in Period 1 by decreasing the protein 15 grams and increasing the carbohydrate by 15 grams, making the dietary formula C. 245 grams, P. 85 grams, F. 85 grams. The carbohydrate equivalent was 6 grams more than that in Period 1. The amount of sugar in the urine decreased from an average of 18 grams to 13 grams for the first 10 days on this diet, and to 9 grams during the second 10 day period. This indicates the utilization of 289 and 293 of the 302 grams of available carbohydrate in the two consecutive 10 day periods, as compared with 278 grams dextrose available in Period 1.

*Period 3.* The patient in Period 2 had demonstrated the ability to utilize 289 grams of dextrose with a protein intake of 85 grams per diem. During the third period, of 46 days, the carbohydrate equivalent was reduced to 279 grams by reducing the carbohydrate and protein in the diet each by 15 grams, making the dietary formula C. 230 grams, P. 70 grams, F. 85 grams. The glycosuria continued at about 10 grams per diem. On January 20, insulin was first given, 20 units being required to prevent glycosuria. We were then able to progressively reduce the insulin to 5 units daily without the recurrence of glycosuria. When administration of insulin was omitted entirely the average amount of sugar excreted daily was 2.5 grams during the last 15 days of this period. This degree of glycosuria was practically constant and showed no tendency to increase. This last period of 15 days seemed to us an adequate control period for an acute experiment, for we recognized that any change to be significant must be marked and definite.

*Period 4.* During this 16 day period the diet was changed to C. 290 grams, P. 70 grams, F. 85 grams. (This increase of 60 grams in carbohydrate was accomplished over an eight day period. The addition of 60 grams of carbohydrate resulted in a daily average of 22 grams of sugar over a 10 day period. Here again there was no tendency for the glycosuria to increase over the last 10 days of this period.

*Period 5.* This period of high protein feeding lasted for 41 days. The extra 60 grams of carbohydrate added in Period 4 was omitted, and 100 grams of protein added, making the dietary formula C. 230 grams, P. 170 grams, F. 85 grams. The available carbohydrate of this diet was almost identical with that in Period 4 and was, for practical purposes, isocaloric. During the first 10 day period of this diet the average daily sugar excretion decreased to 17 grams from the daily average excretion of 22 grams for the last 10 days of Period 4. In the second 10 day period, from May 24 to April 2, the average daily excretion was 25 grams of sugar, and from this point on the severity of the diabetes progressed rapidly: The average from April 2 to April 12 was 33 grams; from April 12 to April 22, 55 grams. On April 22 and 23, 120 grams of sugar was found in the urine, and on April 24, 275 grams, accompanied by thirst and polyuria but no acetone bodies. The patient on this day utilized only 62 of the 337 grams of available carbo-

hydrate in the diet. The patient was now returned to the diet in Period 4.

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#### COMMENT

The addition of an excess of protein to the diet of these two individuals having pernicious anemia did not result in an immediate increase in severity of the diabetes. In M. K., following the addition of 80 grams of protein to her diet, a mild insulin reaction occurred on the second day, causing us to decrease the dose of insulin from 10 to 5 units daily. In R. G., the addition of 100 grams of extra protein and a simultaneous decrease in the carbohydrate of the diet by 60 grams was followed by a diminution in the daily excretion of urinary sugar for 10 days. The addition of excess protein to the diet in the cases reported by Gephart, Aub, DuBois and Lusk (6), Geyelin and DuBois (7), and by Wilder, Boothby and Beeler (8) resulted in the immediate increase in the severity of the disease. We believe it possible that the high carbohydrate content of the diet may have protected our patients from the immediate deleterious effects of the added protein. The carbohydrate content of the diets used by the

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to C. 200 grams, P. 70 grams, F. 85 grams the patient surreptitiously obtained food.

The deleterious effect of protein on the carbohydrate tolerance remains for some time after a return to a high carbohydrate, low protein-fat diet. In 25 days patient R. G. had a tolerance for 229 of 279 grams of available carbohydrate in her diet, while prior to the high protein period her tolerance was 317 of the 339 grams of available carbohydrate. Six months following discharge this patient had regained but little of her original tolerance. Patient M. K. in 25 days following cessation of the period with extra protein utilized 234 grams of 299 grams available, while prior to the high protein period she utilized 229 of 249 grams of available carbohydrate. These figures, while roughly giving one the degree of severity of the disease, are not strictly accurate unless the carbohydrate content of the diets in the two periods are similar. Increasing the amount of available carbohydrate in the diet, provided the increase is by additional carbohydrate rather than protein, often results in an increase in the total sugar utilized. R. G., February 12 to 27, utilized 294 grams of 299 grams available; and with no change in status other than the addition of 60 grams of carbohydrate to the diet utilized, in the period from March 6 to 14, 337 grams of the 359 grams available.

The deleterious effect of a high protein diet remains and is progressive when marked or long continued glycosuria is prevented, and even occurs when glycosuria is absent. M. K., during the high protein diet, was prevented from showing large amounts of glycosuria by constantly increasing dosage of insulin. For 14 days, November 4 to 18, glycosuria was present, but not marked nor accompanied by polyuria. From November 19 to December 2 this patient was absolutely sugar free. Nevertheless this patient showed as much impairment of carbohydrate tolerance as R. G., who was allowed uncontrolled glycosuria.

#### SUMMARY AND CONCLUSIONS

We have studied two subjects having combined pernicious anemia and diabetes over six and nine months of hospitalization. One patient entered the wards in a state of severe diabetic ketosis and in a relapse of the anemia. The other patient en-

tered the wards in a relapse of the anemia and with many psychotic manifestations. Following treatment for the anemia by parenteral injection of liver extract, iron by mouth, and high caloric, high protein diet over a three month period, diabetes developed. The carbohydrate tolerance of both patients was studied on low and high protein diets, with the following conclusions:

1. High protein diets in our two cases markedly reduced the carbohydrate tolerance. This reduction in tolerance cannot be explained by either an increase in calories or by the extra carbohydrate made available from the excess protein.
2. A simultaneous high carbohydrate diet apparently prevented the immediate deleterious effects of the excess protein becoming apparent. Ultimate results were qualitatively similar to that observed in previous studies when low carbohydrate diets were employed.
3. The loss of carbohydrate tolerance as a result of the high protein diet is not temporary. The tolerance remained impaired in one subject for at least 25 days, and in the other for at least six months.
4. Insulin, when given in amounts sufficient to prevent either long continued or severe glycosuria, does not ward off the deleterious effect of excess protein in the diet.

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# BLOOD PRESSURE CHANGES IN NORMALS AND IN HYPERTENSIVES AFTER INTRAVENOUS EPINEPHRINE AND HISTAMINE

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The conception that peripheral vascular irritability may be an important factor in the mechanism of production of hypertension has led to a search for vascular or vasomotor stimuli that would further knowledge both of the physiology of hypertension and of the state of individual patients from time to time. Large numbers of agents, both physical and chemical, have been tried. Of the various chemical stimuli for testing peripheral vasomotor irritability in hypertensive disease, epinephrine has been frequently chosen. In spite of this, there has been lack of agreement concerning the nature of the reactions in hypertension. Jensen (1) reviewed literature and carried out experiments with subcutaneous epinephrine. He found that repetition of tests regularly produced a sharp pressor response, and that the response to the first test was the chief variable. In the normal there was usually a slow systolic rise; in the hypertensives some had little response and others a very strong response on the first test.

About ten years ago a considerable amount of work, reported chiefly in the German literature, was done with injection of epinephrine intravenously. Csépai, Fornet and Toth (2), and Hetényi and Sümegi (3) reported frequent but not constant increased sensitivity to epinephrine in hypertension as judged by the absolute increase in blood pressure following standard doses of epinephrine hydrochloride, usually 0.01 mgm. Jansen (4), using doses of from 0.005 to 0.02 mgm., reported diminished sensitivity in about half the hypertensives, as judged by the amount of elevation of systolic pressure expressed as a percentage of the base systolic pressure. All these investigators observed depressor responses at times in hypertension. Deicke and Hülse (5) judged the type of response rather than its magnitude; in a small series they observed a depressor response in the normal, diphasic response in ne-

phritis and in hypertension with severe kidney damage, and no change in blood pressure in uncomplicated essential hypertension following injections of 0.005 mgm. They regarded pressor response to this dose as indicative of heightened sensitivity to epinephrine and believed it characteristic of patients with renal damage. Hess (6) chose as a criterion the minimal active dose of epinephrine, which gave as a rule negative or diphasic blood pressure curves; she reports lower minimal doses in hypertension, although there was considerable overlap into the normal minimal dose range. She considered as normal sensitivity 0.000005 to 0.000010 mgm. per kgm. body weight for the minimal active dose; in a 60 kgm. subject this would mean doses of 0.0003 to 0.0006 mgm. Her sensitive individuals reacted at times to doses as low as 0.0000025 mgm. per kgm. Szondi (7) also pointed out the difficulty in drawing conclusions from the direction of the blood pressure change following the injection of a single dose of epinephrine, and observed that in most subjects a depressor response was obtained if the dose was made low enough. He agreed with Hess in the use of minimal depressor doses as a measure of sensitivity to epinephrine.

The various criteria for judging blood pressure reactions to intravenous epinephrine may be summed up under the following heads: (1) type of response; (2) size of dose required to produce any effect or to produce stated effects; and (3) magnitude of response.

We have carried out 102 experiments on 63 subjects in which we have attempted to observe the response to intravenous epinephrine hydrochloride from all three standpoints. Histamine dihydrochloride also was administered in 86 experiments in the same fashion. Of the 63 subjects 25 had primary hypertension, 9 had glomerulonephritis, 16 were normal, 7 had normal blood pressures at the time of the examinations but



were known to have hypertension at times, 3 had arteriosclerosis, and 3 had hyperthyroidism.

#### METHOD

The subject lay at ease on a couch, and a needle connected with a syringe containing salt solution was inserted into a vein in the antecubital fossa. The blood pressure was observed every 30 seconds in the opposite arm by the auscultatory method, throughout the experiment. When the blood pressure had reached a constant level, usually after 15 to 25 minutes, a measured dose of epinephrine hydrochloride in saline solution was injected at such a speed as to require as nearly as possible 30 seconds for the injection. A base line was generally reached again after 4 to 8 minutes so that another dose could be administered through the same needle. The epinephrine solutions were freshly prepared either from solid tablets or from 1:1000 solution in dilutions of 1:10,000 to 1:1,000,000. It was ascertained on three subjects that the reactions were the same whether the tablet or the commercial solution were the source of the epinephrine. The doses regularly used were 0.0010, 0.0015, 0.0025, 0.0040, 0.0060, 0.0100, and 0.0150 mgm.; larger and smaller doses were used when necessary to determine minimal and maximal depressor doses. The order in which various doses were given was varied in some experiments; it did not appear to affect the results. We found it convenient, as a rule, to work from small to large doses, because of psychic stimulation from palpitation after the larger doses. The histamine was used at times before and at times after epinephrine. It was prepared from 1:1000 solution of the dihydrochloride in the same dilutions as epinephrine.

#### RESULTS

In evaluating the blood pressure results some arbitrary standards were set up for the sake of uniformity. Changes of 2 mm. were regularly disregarded. Changes of 4 mm. were counted if they were confirmed by repetition or if a slightly greater dose produced a slightly greater change in the same direction, provided the base line were satisfactory. Changes of 4 mm. were also counted as significant when they represented the short arm of a diphasic response. Otherwise

changes of 6 mm. or more only were held to be significant.

*Type of response.* The type of reaction followed the same general pattern in all groups. With few exceptions the least effective dose produced a fall in both systolic and diastolic pressures. As the dose was increased a pressor phase appeared following the depressor phase. With further increase in dosage the depressor phase grew less in magnitude until it was no longer found, while the pressor reaction increased. Depressor reactions were found at some dosage in 89 of 102 experiments. They were transitory and occurred 30 seconds after the cessation of injection. Usually the pressure was at the base line or higher at the end of another 30 seconds. The pressor phase began 30 to 60 seconds after the cessation of injection, sooner with large than with medium doses. It was usually at its highest 60 or 90 seconds after the injection, and the base line was reached in 1 to 5, usually about  $2\frac{1}{2}$ , minutes. The diastolic pressure generally followed the direction of the systolic, except that where a pressor response was obtained the diastolic usually rose and fell about 30 seconds before the systolic. This was true even when the systolic rise was preceded by a fall.

The 13 experiments in which depressor response was not found were distributed as follows: primary hypertension, 7 of 41 experiments; nephritis, 5 of 26; transitory hypertension, 1 of 8; normals and others, none of 27. It is possible that depressor reactions were present at times and not observed because of their transitory character.

*Size of dose.* The dosage level at which the various phases of the response were obtained varied within wide limits. Figure 1 gives frequency curves for two groups, those in whom the base systolic pressure is below 150 mm., and those in whom it is 150 mm. or more. The minimal effective dose was found at 0.0010 or less, 0.0015, and 0.0025 mgm. with practically equal frequency; in 15 experiments it exceeded those figures. The hypertensive group included a slightly greater spread at each end, but presented no significant difference in general trend. The spread is still more apparent in the curve for minimal depressor doses, as 11 of the 13 experiments in which the minimal dose was pressor were among the hypertensive group. There was no significant

difference between the minimal pressor doses of the two groups. In the curves of maximal doses at which depressor response was observed, there is a mode at 0.0040 mgm. in the lower and a mode at 0.0025 in the higher pressure group. The latter group, however, contains also more experiments in which the maximal depressor doses were unusually high, and these tend to counterbalance the difference in modes.

depressor doses did not appear to vary significantly from group to group. The distribution of the dosages in the transitory hypertension experiments appears to be well within the normal range for each of the effects.

There was a considerable degree of variation in the responses of a single individual on different days for no demonstrable reason. In one subject, a man suffering from acute glomeruloneph-

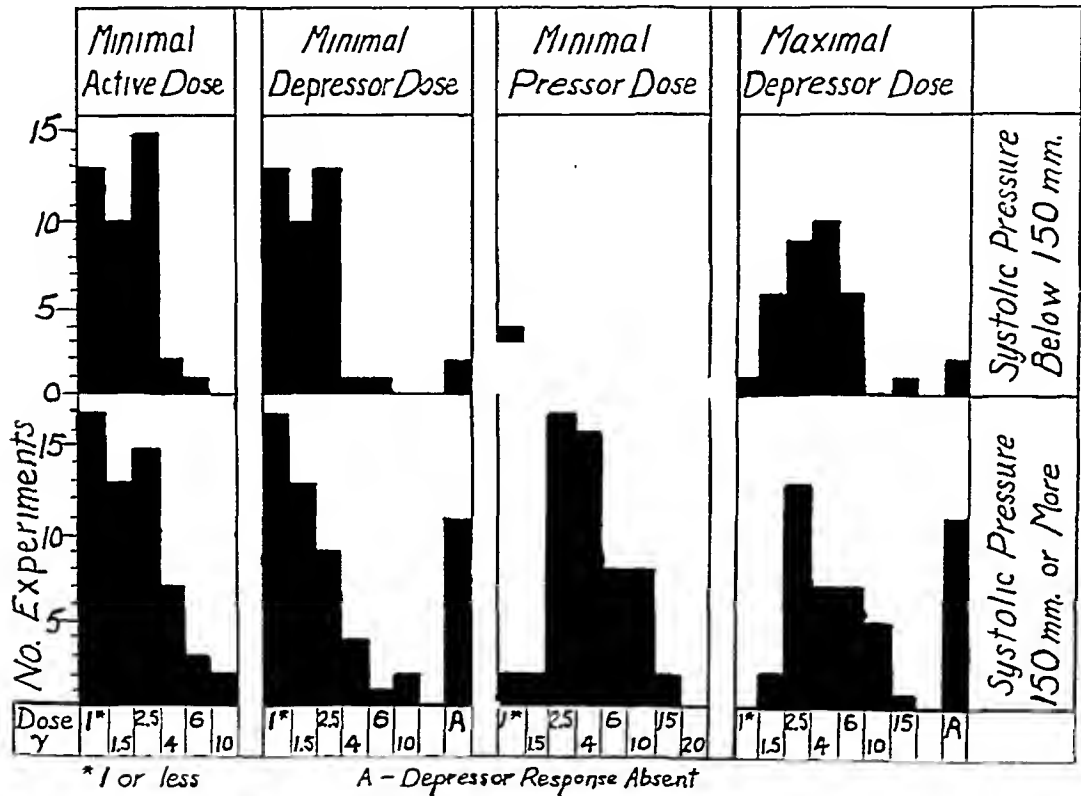


FIG. 1. FREQUENCY WITH WHICH MINIMAL ACTIVE, MINIMAL DEPRESSOR, MINIMAL PRESSOR, AND MAXIMAL DEPRESSOR DOSES OF EPINEPHRINE HYDROCHLORIDE WERE FOUND AT VARIOUS LEVELS IN SUBJECTS CLASSIFIED ACCORDING TO SYSTOLIC PRESSURE.

Similar frequency curves for the diagnostic classifications are shown in Figure 2. The minimal activity dose was found more frequently in primary hypertension at lower, and in nephritis at higher, levels than in the normal. In some subjects with primary hypertension, however, there were also very large minimal doses. The minimal depressor doses varied in the same way as minimal active doses. The minimal pressor doses tended to be slightly higher in primary hypertension than in the other groups. The maximal

depressor doses did not appear to vary significantly from group to group. The distribution of the dosages in the transitory hypertension experiments appears to be well within the normal range for each of the effects.

*Magnitude of response.* When blood pressures are observed at intervals, rather than continuously, any conclusions with regard to magnitude of change depend on the assumption that the peaks of the blood pressure curves are as likely to be observed in one subject as in another, since it is

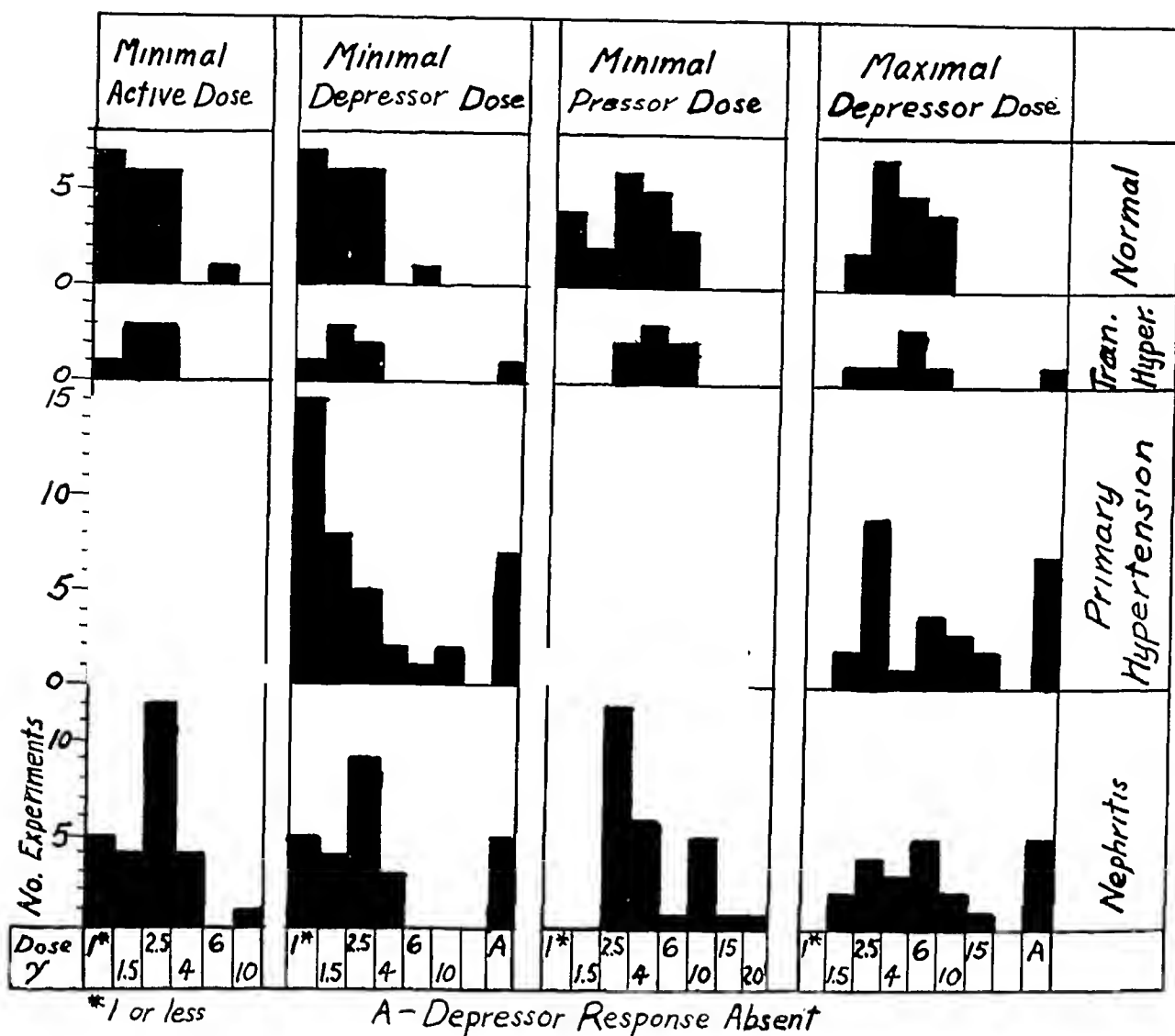


FIG. 2. FREQUENCY WITH WHICH MINIMAL ACTIVE, MINIMAL DEPRESSOR, MINIMAL PRESSOR, AND MAXIMAL DEPRESSOR DOSES OF EPINEPHRINE HYDROCHLORIDE WERE FOUND AT VARIOUS LEVELS IN SUBJECTS CLASSIFIED ACCORDING TO DIAGNOSIS.

*Tran. Hyper.* refers to transitory hypertension.

TABLE

*Magnitude of changes in systolic pressure observed after intravenous administration of epinephrine hydrochloride*

Dose	Initial systolic pressure	Frequency of falling pressure	Mean initial systolic pressure	Mean fall	Mean fall	Frequency of rising pressure	Mean initial systolic pressure	Mean rise	Mean rise
mgm.	mm. Hg	experiments	mm. Hg	mm. Hg	per cent	experiments	mm. Hg	mm. Hg	per cent
0.0015	100-139	21	123	6.9	5.6	6	128	7.0	5.5
	140-179	15	160	8.3	5.2	3	151	6.7	4.4
	180-230	17	205	14.6	7.1	7	203	9.7	4.8
0.0025	100-139	25	123	7.7	6.3	17	124	5.9	4.8
	140-179	18	156	8.6	5.5	16	157	6.8	4.3
	180-230	21	201	10.2	5.1	17	201	9.0	4.5
0.0040	100-139	17	120	9.4	7.7	26	123	7.2	5.9
	140-179	18	159	9.3	5.9	18	159	7.3	4.6
	180-230	16	206	13.0	6.3	22	200	11.7	5.9

obvious that they cannot be observed consistently. In spite of this qualification it is possible to judge the magnitude of response in a general way.

The series of responses to each single dose was divided into three groups according to base-line systolic pressure, the divisions being made at 140 and 180 mm. The averages of all pressor responses and of all depressor responses within these groups were compared both in absolute magnitude and as fractions of the mean systolic pressures within the groups. The figures indicated in Table I for 0.0015, 0.0025, and 0.0040 mgm. are typical. It will be seen that while the average rise or fall is greater from the higher base line, its magnitude, expressed as a percentage of systolic pressure, is practically the same in hypertensives as in those with normal blood pressure.

**Histamine.** When a sufficient dose of histamine dihydrochloride was given the systolic and diastolic pressures fell. Frequently a sharp fall was followed by a transient rebound to 4 or 6 mm. above the previous base line. The depressor response, when present, was found 30 seconds after the cessation of the injection, and had disappeared in another 30 seconds. It was regularly accompanied by a flush; doses just insufficient to cause demonstrable fall in blood pressure were also, as a rule, productive of flushing.

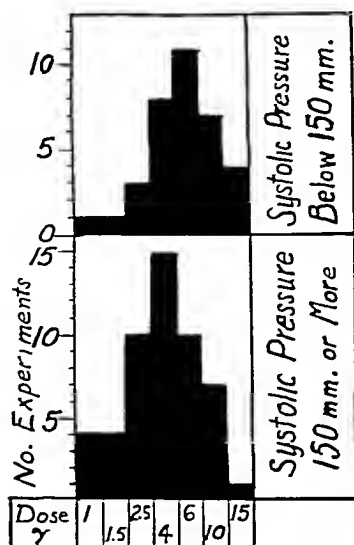


FIG. 3. FREQUENCY WITH WHICH THE MINIMAL DEPRESSOR DOSE OF HISTAMINE DIHYDROCHLORIDE WAS FOUND AT VARIOUS LEVELS IN SUBJECTS CLASSIFIED ACCORDING TO SYSTOLIC PRESSURE.

The least dose at which significant depression of the systolic pressure was observed varied from 0.0010 mgm. to 0.0150 mgm. The frequency with which the subjects reacted to the various doses is indicated in Figure 3. It is evident that on the average histamine produces a fall in blood pressure at slightly smaller dosage when the blood pressure is high than when it is low. The overlap between the hypertensive and non-hypertensive minimal dose ranges is extensive.

Scatter graphs, in which the minimal depressor dose of epinephrine was plotted against the minimal depressor dose of histamine for the same subject at the same sitting, showed no apparent relationship. We have not attempted to form any conclusions from the magnitude of response to histamine because of the extreme rapidity of the changes and the marked variation of the magnitude of change on even immediate repetition.

#### DISCUSSION

The differences that we have noted between hypertensive and normal individuals in their response to epinephrine and to histamine have in no case been striking. For both substances the type of response is the same in the one group as in the other. The doses at which various responses to epinephrine were obtained show only slight differences. A slightly more definite difference as regard usual size of dose was observed in the histamine experiments, but there was no clear-cut separation of groups on the basis of dose required. Differences in magnitude of response appear to be insignificant. It is thus evident that neither epinephrine nor histamine, in the way we have used them, is the ideal vasomotor stimulus for the study of hypertensive and "pre-hypertensive" states.

The observed similarity admits of several interpretations. It is possible that the effects of both are so complicated by cardiac, peripheral vascular, pulmonic vascular, and nervous factors as to make the blood pressure changes they produced valueless as measures either of vasomotor irritability or of vascular tonus. It may be that the method is too gross to detect small differences in irritability or tonus. Furthermore, it is possible that the mechanism of blood pressure changes after such stimulation is different from the mechanism of blood pressure regulation under ordinary condi-

tions. Finally, it is possible that the vasomotor irritability is actually measured in a rough way, but that the irritability of the hypertensive individual is essentially the same as that of the normal. Lacking adequate evidence as to the mechanism of the blood pressure changes, particularly of the depressor responses to epinephrine, we have not attempted to decide the cause of the major resemblances and minor differences in the reactions of normals and of hypertensives.

#### SUMMARY

The blood pressure changes were observed in normal and in hypertensive subjects following the intravenous injection of varying doses of epinephrine and of histamine. Hypertensive subjects reacted to these substances in the same general way as did those with normal blood pressure. With minor variations the doses of epinephrine required to produce effects in the normals were about the same as those required to produce the same effects in hypertensives. The blood pressure changes after epinephrine injection were approximately equal when expressed as a percentage of systolic pressure. Hypertensives reacted

on the whole to slightly smaller doses of histamine than normals.

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# PNEUMONIA DUE TO PNEUMOCOCCUS TYPE XIV (COOPER) AND ITS TREATMENT WITH SPECIFIC ANTISERUM<sup>1,2</sup>

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Because of the anatomical similarity of the lesion in the lung, diseases caused by different types of pneumococci have been described together as a single disease. The difference in the severity of pneumonias due to different types of pneumococci was recognized in the monograph of Avery, Chickering, Cole and Dochez (1). Prior to studies made possible by the Cooper classification of the pneumococci, most physicians believed that the pneumonias due to pneumococci of Group IV (Avery) were less severe than the recognized Types I, II and III.

The specific types of pneumococci are serologically distinct though most of them cannot be distinguished by differences in colony form or cell morphology. They are distinguished by means of the specific reaction with antibodies produced in rabbits or horses. Our studies have now progressed to a point where the number of cases each due to a single type of pneumococcus is sufficiently numerous to warrant a tentative statement of the kind of clinical reaction or disease caused by certain of these types.

Types behave differently in age incidence, blood invasiveness, complications and severity of illness, and in the character of the consolidation as inferred from the physical signs and radiograms. In some instances the general picture may be so definite that the type of invading pneumococcus has occasionally been correctly suspected or guessed before an examination of the cultures had been made.

<sup>1</sup> Read before the Section of Medicine, New York Academy of Medicine, October 16, 1934.

<sup>2</sup> Our appreciation is expressed to loyal coworkers. Serum was produced through a grant from the Altman Foundation, Inc., and the children were studied, in part, through financial assistance from the Commonwealth Fund. The Metropolitan Life Insurance Company assisted this study. We are grateful to Miss Cooper for checking the correctness of typing, in cultures sent to her.

We are about to describe the pneumonias due to *Pneumococcus* Type XIV of whose origin Miss Cooper says:

"After the differentiation of Types IV to XIII, a number of strains hitherto unclassified were selected for the production of additional antisera (in rabbits) for typing, among which were strains isolated from:

*Patrick Thompson*: Age 39, Harlem Hospital, November 26, 1928, severe illness; recovered. Strain from positive blood culture November 28, 1928.

*Catherine Murray*: A child. Harlem Hospital. December 23, 1928. Died. Strain from positive blood culture December 23, 1928.

*Clark*: Age 2¾. Bellevue Hospital, January 5, 1929. Moderately severe illness; recovered. Strain from throat swab January 5, 1929."

"When agglutination tests were carried out with these antisera and the various stock strains, the three strains above mentioned were found to be similar and these, along with other strains agglutinated by these antisera, were termed Type XIV."

*Frequency of occurrence.* This study is principally based on our experience with 127 cases of pneumonia due to *Pneumococcus* Type XIV observed at Harlem Hospital in the lustrum from July 1, 1928 to June 30, 1933. The adults (43 patients older than twelve years) were observed on our Pneumonia Service, and the children (85) on the Pediatric Service through the generous permission of Dr. Morris Gleich.

*Pneumococcus* Type XIV is one of the most frequent invaders of children, causing 16.1 per cent of the 547 pneumococcus pneumonias in our series. Among 2546 adults suffering from pneu-

mococcus pneumonia it was the cause in 2.6 per cent. Raia, Plummer and Shultz (2) found Type XIV in 20 of 182 cases of pneumonia in children at Bellevue Hospital, with 10 per cent deaths; 15

females. Among 43 adults, 31 were males. This also occurs among children (among 85 children attacked, 46 were males) and even among the infants under one year of age; among 51 infants

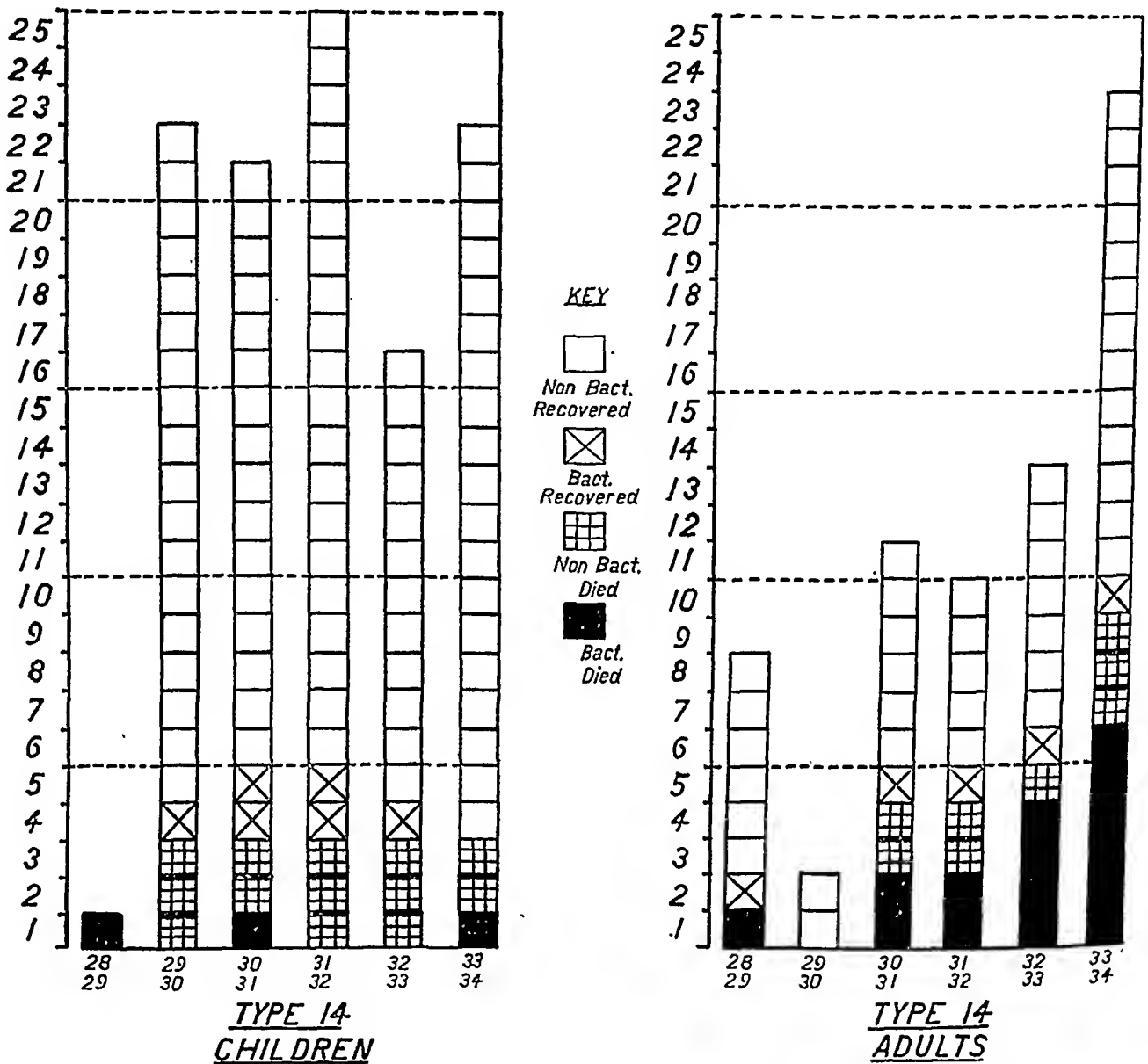


FIG. 1. PNEUMONIA DUE TO PNEUMOCOCCUS TYPE XIV.

Annual incidence of cases and outcome in each of six years. Children and adults separately.

of the cases were in children under three years. Nemir (3) who continued their observations, had 68 cases of Type XIV among 276 children, with a mortality of 18 per cent in infants under two years. Kereszturi and Hauptmann (4), at the Fifth Avenue Hospital, found a 15 per cent incidence of Type XIV among children; they had 17 cases with 3 deaths (17 per cent).

*Sex and age.* Pneumococcus Type XIV, like other pneumococci, attacks many more males than

attacked, 32 were males. Among children the pneumonias occurred most frequently in the first year. (See Figure 3.)

In Figure 2, which contrasts, in the five and one-half year period from July 1, 1928 to December 31, 1933, the frequency of Type I and Type XIV by year of age, it is shown that before puberty Pneumococcus Type I and Pneumococcus Type XIV are about equally frequent, and together they cause about 31.3 per cent of the

pneumococcus pneumonias in children. There is a marked difference in their selection, however; whereas Type XIV causes one-fifth of the pneumococcus pneumonias during the first three years,

Among adults, Type XIV is increasing in frequency among our cases, as may be seen in the Figure 1 which presents the cases separately for each of six years. Last year there were 23 cases.

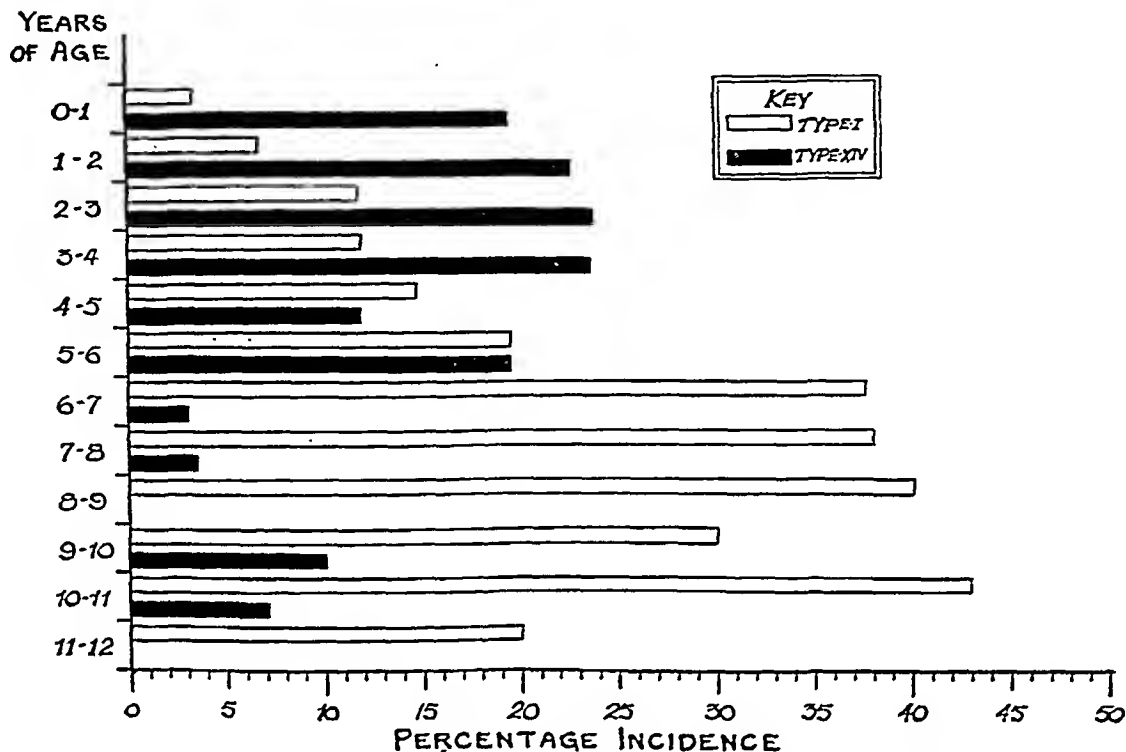


FIG. 2. COMPARISON OF PERCENTAGE INCIDENCE OF TYPE I AND TYPE XIV BY YEAR OF AGE AMONG 547 PNEUMOCOCCUS PNEUMONIAS IN CHILDREN.

July 1, 1928 to December 31, 1933.

after age six it occurs less frequently than Type I. The childhood mortality for Type XIV is much greater than for Type I. For Type I it is 6.4 per cent; for Type XIV, 16.1 per cent. Children of four or five years of age either meet new exposures in the street, the day nursery and the school, or have a changed susceptibility which latter fact may account for the difference in the organism causing the pneumonias. After infancy Type XIV surrenders its dominance to Type I though it does not yield its lead in fatality.

It is significant that our figures agree with the contemporaneous studies of Nemir (3) and Kereszturi and Hauptmann (4) in different sections of the city, and support Nemir's conclusion that Type XIV is the predominant type for infants, and Type I the predominant type among older children.

In the lustrum 1928 to 1933, it produced a high mortality and was most frequently encountered in the fourth decade where it was especially fatal (Figure 4).

*Anatomical form.* Lobar pneumonia, as determined by localization of the consolidation in a single lobe or lobes (usually confirmed radiographically) was more frequently encountered in children than bronchopneumonia (37 among 51); this occurred even in infants under one year. Only three of the 17 instances of bronchopneumonia occurred after the first year (Figure 3). In adults all the cases were of lobar type. As seen in the radiogram, an entire lobe was usually involved and the shadows were not dense. The interlobar fissure was often sharply outlined on the side of the lesion.

*Onset.* The onset was not characteristic. In



children, vomiting was frequently noted at the onset. Among 43 adults only three cases had much higher incidence, 3 among 20 children (15 per cent) in their Bellevue series which preceded

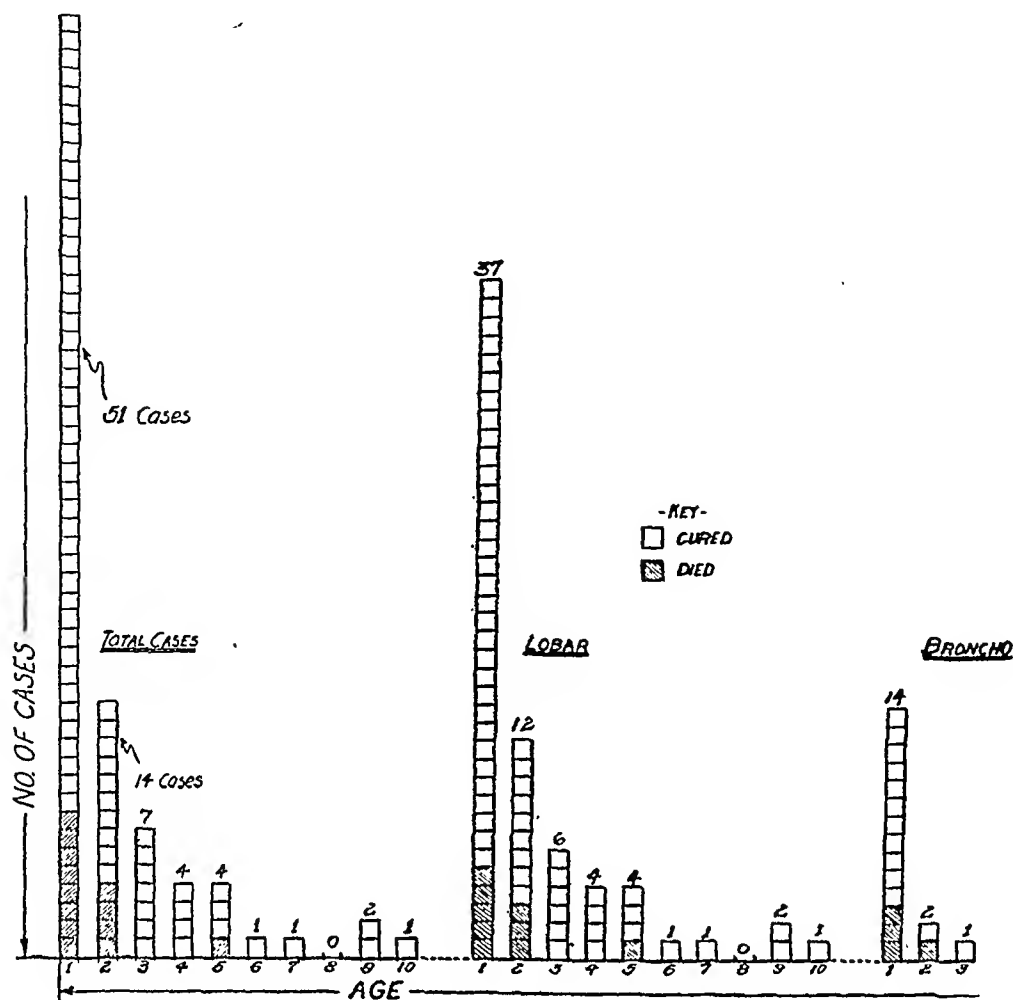


FIG. 3. DISTRIBUTION OF AGE INCIDENCE AND MORTALITY.

Pneumococcus Pneumonias Type XIV (Cooper). Children. 1928-1933.

neither rigor nor chest pain at the onset. Rigor and chest pain ushered in the illness in 24 cases, pain in 15 and rigor in 1. With the invasion, 29 cases had cough, 7 had vomiting, 7 had shortness of breath, 14 had headache and 20 fever. The onset was preceded by cold in the head in 8 cases, and by cough in 7 cases.

*Pleuritic involvement.* Pleuritic involvement as determined by x-ray or physical signs was extremely frequent, occurring in 20 cases out of 85 children, and in 30 among 43 adults.

The occurrence of empyema in the adults (3 out of 43 cases with 3 deaths) is high. The incidence of empyema in children, in spite of the pleural involvement, was not high in our series or in that of Nenir. At Harlem Hospital there was one empyema among 85 cases, and at Bellevue Dr. Nenir reported one empyema among 68 children. Raia, Plummer and Shultz (2) found a

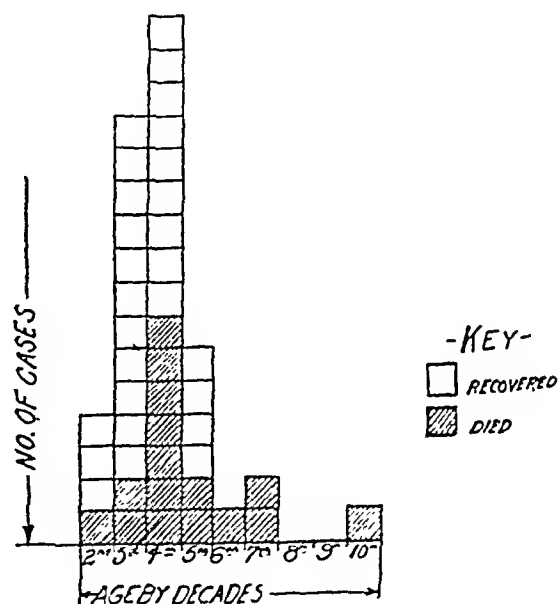


FIG. 4. AGE AND MORTALITY DISTRIBUTION.

Pneumococcus Pneumonias Type XIV (Cooper). Adults. 1928-1933.

the studies of Nemir, and Kereszturi and Hauptmann found 3 among 17 children (18 per cent) at the Fifth Avenue Hospital.

*Lung suction.* Because the determination of type was frequently accomplished by the routine technic of transthoracic lung suction performed on our service, we present the cases of pleurisy and empyema in a table (Table I) classified into

more than twice as fatal in adults (69 per cent) as in children (28 per cent). The death rate in cases where the blood cultures were found to be negative is also greater in adults (23 per cent) than in children (14 per cent).

In some instances only broth cultures were positive or only a few colonies were present on the plates; these cases recovered spontaneously.

TABLE I

*Lung suction in diagnosis of pneumococcus pneumonia Type XIV (Cooper) children and adults, 1928 to 1933*

	Positive lung suction cases		Negative lung suction cases		Total lung suction cases		Non-lung suction cases	
	Children	Adults	Children	Adults	Children	Adults	Children	Adults
Total cases.....	26	13	13	6	39	19	13*	2*
Total deaths.....	4	5	3	1	7	6	33†	22†
Percentage of deaths.....	15	38	23	17	18	32	6	10
Cases with bacteremia.....	2	5	2	2	4	7	13†	42†
Cases treated with serum.....	10	3	2	0	12	3	18†	45†
Cases first typed from lung suction..	13	8					3	6
							1	3

\* Cases with low temperatures.

† Cases with high temperatures.

‡ Based on both groups.

those which had been and those which had not been subjected to this procedure, with the fatalities for each group. The part that lung suction played in the typing is also shown. In half the cases the type was first determined in this way. The mortality was not increased by lung suction. It seems proper to reject from comparison those patients convalescent and with a low temperature by the time the result of the pharyngeal culture was returned, and in whom a lung suction was accordingly unnecessary for diagnosis and was not done.

*Sputum.* The sputum was studied in respect to character. It was noted that thick, prune juice and rusty, tenacious sputum was associated with an unfavorable outcome.

*Blood count.* Blood counts varied in no way from those in pneumonia of most other types.

*Bacteremia.* Bacteremia was found among 8 of the 85 children and was fatal twice. Thirteen among 43 adults had positive blood cultures and 9 of them died. The incidence of bacteremia was more than three times higher in adults (30 per cent) than in children (9 per cent). Its influence on mortality was very marked. Bacteremia was

In many cases, cultures were positive in broth only and colonies did not appear on the plates in later cultures. In some cases there was an increase in colonies which frequently became overwhelming in a short time. In one adult, M. L., the blood became sterile after 15,000 units of specific antiserum was administered. In one case there were many thousands of colonies for a number of days before death.

*Specific immunity.* The presence of immunity was tested by the presence of agglutinins by the stained slide technic. In this study it was found that in 12 patients (adults and children) in whom agglutinins were detected, none died; they may have appeared spontaneously, have been induced by the injections or have been passively transferred in them. Eight children who were given serum recovered although agglutinins were not demonstrated in their blood during the administration of serum or subsequently. One child died among 15 children who were not given serum and in whose blood agglutinins were not found. Among the adults, 2 patients died who had been given serum but in whose blood agglutinins were not found. Two of the 7 cases which did not re-



seventeen days, and one patient died on the eighteenth day.

**Termination.** Among 64 adult cases (1928 to 1934) 40 recovered; of these, 28 (44 per cent) terminated by lysis, and 12 (19 per cent) by crisis. Among 83 children (1928 to 1933) 15 (18 per cent) terminated by crisis, 53 (66 per cent) by lysis, and 13 died.

**Complications, associated diseases and menacing symptoms.** The great incidence of menin-

times. Once, *Pneumococcus* Type XIV was cultured; two of the twelve cases not cultured died. Acute gastroenteritis occurred once in a fatal case. Acute tonsillitis and a Vincent's stomatitis occurred in children who recovered. Rickets was present in four children. Two of the three infants who were greatly malnourished died. Four children who showed marked anemia and received whole blood intramuscularly recovered.

A large number of cases required oxygen, 47

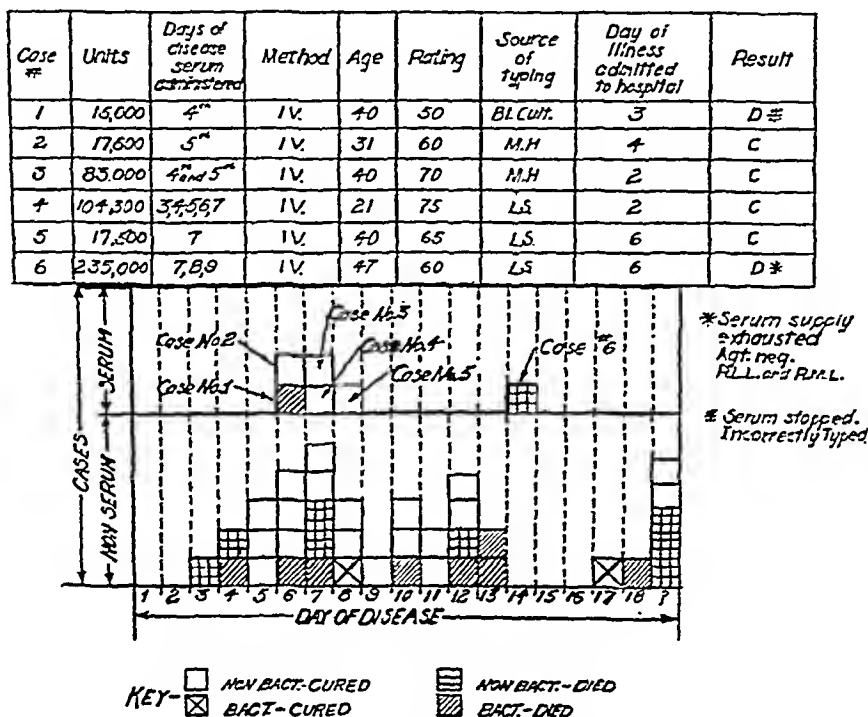


FIG. 6. DAY OF TERMINATION AND OUTCOME.

*Pneumococcus* Pneumonias Type XIV (Cooper). Serum and non-serum. Adults. 1928-1933. I.V.=intravenous. M.H.=mouse heart. L.S.=lung suction. C=cured. D=died.

gitis among the children (3 among 85 cases) is noteworthy. When pleurisy was present it added to the gravity of the disease. Four of 20 children, and nine among 30 adults, who had this symptom died. Pericarditis was fatal whenever it occurred, once in a child and three times in adults. Pyarthrosis of the shoulder occurred once. An associated fatal *B. influenzae meningitis* occurred once. Pulmonary gangrene resulting from an associated *Staphylococcus albus* lung abscess was found postmortem in one instance. Among the children, otitis media occurred fifteen

out of 85 (56 per cent) among the children, and 18 among 43 adults (42 per cent). Delirium and distention occurred frequently. Seven of the 9 delirious adults died. Three fatal cases had required restraints. Two of the fatal delirious patients had a bacteremia. Five children were distended; three of these had an associated anoxia, and only these three died. Nine of the 15 adults who were distended were also anoxic; five of the 9 who were anoxic had a positive blood culture; these and three others died. Those without anoxia recovered. Icterus occurred once in a fatal

case. Severe hiccough occurred once, with ultimate recovery. Fatal pulmonary edema was, as usual, frequently associated with bacteremia. The temperature, pulse and respiration were no different than in similar severe cases among other types.

In contrast with its virulence for human beings, it is significant that none of the cultures of Type XIV from children or adults sent to Miss Cooper from our service or from Bellevue Hospital have so far been sufficiently virulent for white mice to permit titration of the potency of therapeutic sera by protection tests.

One thousand to one hundred thousand organisms are required for the minimum lethal doses of the Type XIV strains thus far examined, while two or three organisms of the strains of other types used as test cultures in protection tests are fatal for mice.

*Electrocardiographic changes.* We shall report elsewhere, with Lowen, the electrocardiographic findings in 140 patients suffering from pneumonias of different types of pneumococci. Only two patients, both with pneumonia due to *Pneumococcus* Type XIV, developed, during convalescence, coving of the T wave in Leads I and II which was indistinguishable from that found in coronary artery disease.

*Description of disease produced by *Pneumococcus* Type XIV.* We may sum up our observations by describing the pneumococcus pneumonias due to *Pneumococcus* Type XIV as a disease especially common in very young children, producing usually a lobar pneumonia, even in infants. Among adults it frequently invades the blood, causing a severe disease with pleurisy, pericarditis and meningitis. In children it is of prolonged duration and, at times, it is rather tardy in the production of agglutinins (demonstrable by the slide technic). The production of the agglutinins is, as in other types, usually of good omen.

*Serum.* In view of the very great fatality and blood invasiveness of this disease, it seemed desirable to attempt to produce a therapeutic antiserum. This was produced under the direction of Dr. Park at the Bureau of Laboratories of the Department of Health, with financial assistance from the Altman Foundation and the Littauer Fund. Since the beginning in April, 1929, of the

attempt to produce antisera for Type XIV, twelve horses have been allotted to this purpose. The difficulty in obtaining antiserum is exemplified by the fact that two of the horses died after having been injected for four months, one after five months and one after ten months. No serum suitable for therapeutic use was obtained from these horses. Suitable serum was obtained from two horses which were injected for a period of ten months and from two others injected for seventeen months. Four horses are being injected at the present time. Three have been injected for ten months and the injections of one horse have just been started. Serum for therapeutic use is being collected from two of the horses.

TABLE II  
*Serum treatment in adults and children,  
*Pneumococcus* Type XIV (Cooper)*

From 1928 to 1933	Non-serum			Serum		
	Cases	Deaths	Per cent	Cases	Deaths	Per cent
Adults (43)...	37 12*	14 8*	38 67*	6† 1*	2 1*	33 100
Children (85)...	70 7*	12 2*	17 29*	15 1*	1 0*	7 0*
Total (128)...	107 19*	26 10*	24 53*	21 2*	3 1*	14 50*
Children from July 1, 1928 to July 1, 1934						
Under three years (82)...	63 5*	12 2*	19 40*	19 2*	2 1*	11 50*
Birth to 12 months (36)...	27 1*	5 0*	19 0*	9 1*	0 0*	0 0*
Three years and over (25)	20 2*	1 0*	5 0*	5 0*	1 0*	20 0*

\* Asterisk indicates cases with positive blood culture.

† Including two incompletely treated because of lack of serum—fatal. Only one case (not included) was treated in 1934—recovered.

Eleven preparations of concentrated refined antiserum have been prepared. The method of concentration used was the same as that used for Type I antiserum.

Pending the discovery of a more virulent

strain, the potencies of the Type XIV antisera are tested by titrations of their precipitin and agglutinin content and are calculated in comparison with antisera for other types which have been more completely tested. Experience with this antiserum has not been sufficiently great to permit final conclusions as to its value. It has been possible to terminate the disease in some of the cases by intramuscular injections. The failures

there were no deaths. In adults, 4 cases adequately treated, recovered. Two insufficiently treated (in one case because the supply of serum was exhausted) died.

The statistics suggest that treatment by antiserum was helpful. Termination of disease occurred after the administration of sufficient antiserum to effect humoral immunity, as shown by the demonstration of agglutinins in the blood.

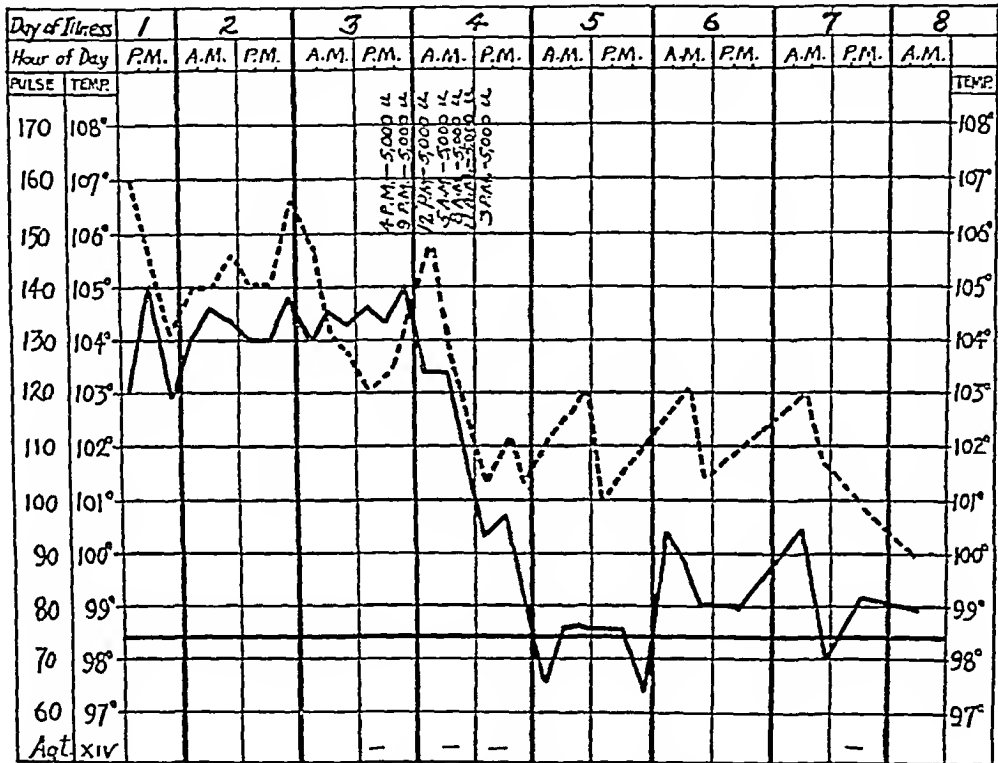


FIG. 7. CASE M. T. FEMALE, 5 YEARS OF AGE.

Sputum, Type XIV, lung suction Type XIV, blood culture negative. X-ray, pneumonia right lower lobe. Distended. O<sub>2</sub> per nasal catheter. Agglutination remained negative. Solid line, temperature; broken line, pulse rate.

appear to have been due to insufficient serum. When little serum was available the sickest cases were chosen; at other times alternate cases were given antiserum. The results are seen in Table II. In children under three, there were 63 cases with 12 deaths (19 per cent) in the controls; of the 19 who were treated, there were 2 deaths (11 per cent). There were 27 cases under a year who did not receive antiserum; 5 died, a mortality of 19 per cent. In the same age group, 9 cases received antiserum in the thighs or buttocks and

The following case histories illustrate either the normal course of the disease or reveal the fall of temperature which occurred, in most cases, at the same time that agglutinins appeared when antiserum was administered either early or later in the disease.

#### CHILDREN

1. H. G., female, 3½ years. Lobar pneumonia, left lower lobe. The temperature was elevated, 103° to 105° F., with a rapid pulse (120 to 140) and accelerated respiration (40 to 60) for a week:

prior to admission. In the oxygen chamber the fever continued, with a crisis of the temperature on the 16th day and of the pulse on the 18th day.

2. M. T., female, 5 years, lobar pneumonia, right lower lobe; distended. Temperature 104° to 105° F., pulse 120 to 170, and respirations 40 to 50. Type obtained by lung suction. Critical termination of fever came on the fourth day after administration of seven injections each 5000 units of serum into

phlebitis left tibial veins developed on eleventh day. Death occurred on twelfth day.

4. F. F., male, 35. Bacteremia. Extensive consolidation was present. Death on 5th day. No agglutinins appeared.

5. M. P., female, 40. Right lower lobe involved. Temperature 104° F., pulse 120. There was a critical termination after three intravenous doses of 5000 units of antiserum, four hours apart, on the sixth

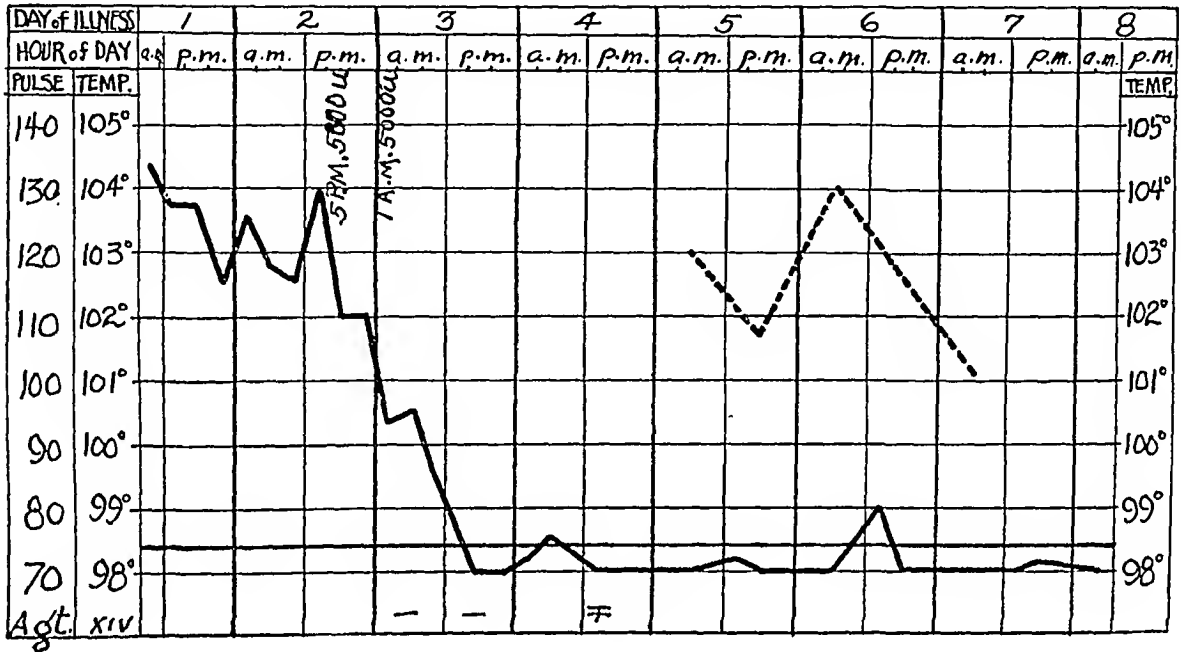


FIG. 8. CASE N. S. FEMALE, AGE 11 MONTHS.

Marasmus. O<sub>2</sub> per nasal catheter. Radiograph negative. Fluoroscopy—increased hilum shadow and slight opacity left base. Lung suction Pneumococcus Type XIV, laryngeal culture Type XIV, blood culture negative. Solid line, temperature; broken line, pulse rate.

the buttocks at three hour intervals. No agglutinins appeared. (See Figure 7.)

3. N. S., female, 11 months, marasmus. Admitted on the first day. Treated on the second day. Temperature 102.6° to 104° F. Respirations 64. Crisis came on the third day, with agglutination  $\mp$  on the fourth day. Type was obtained by lung suction. Slight opacity was noted with fluoroscope. This child received two intramuscular injections (5000 units) twelve hours apart on second and third day. (See Figure 8.)

ADULTS

1. E. C., male, 30. Right upper lobe involved. Agglutination appeared on the fifth day. Temperature became normal on sixth day. Type obtained by lung suction. No serum given.

2. M. N., female, 37. Left lower lobe. Agglutination evident on seventh day; became stronger on ninth day, when the temperature fell to normal.

3. F. H., male, 45. Temperature had fallen before admission. Strong agglutination was present on tenth day. Auricular fibrillation, and thrombo-

day. Agglutinins were not demonstrated. Pneumococcus Type XIV was recovered from sputum and lung suction.

The death of one child was due to anaphylactic shock from the administration of a 5 cc. dose of antiserum given into the jugular vein. We are satisfied that this is an excessive initial dose of serum by vein for children. We now limit the initial intravenous dose of serum in children to 1 cc. or we use it intramuscularly. In adults the antiserum for Type XIV pneumococci seemed to produce more reactions when given intravenously than antiserum for other types with which we are now making observations.

In view of the frequency, the prolonged duration, the relatively high mortality rate (especially in the bacteremias among adults) of Type XIV infections, and the suggestive results achieved in treatment by specific antiserum, it seems impor-

tant to produce and administer antiserum and to attempt to increase its potency and to endeavor to refine it.

#### SUMMARY

To summarize, *Pneumococcus* Type XIV is a virulent organism for human beings, selecting by preference infants and young children, in whom the pneumonias are usually of long duration. It has increased in frequency among adults admitted to our service; in these subjects it is especially prone to invade the blood and to prove fatal. It apparently involves the pleurae pericardium and meninges with greater frequency than many other types of pneumococci. Upon recovery, agglutinins appear. Protective antibodies, including agglutinins, can be transferred from horses injected with this pneumococcus. In many

of our cases there was evidence that when sufficient antiserum was given there occurred deferescence and earlier recovery.

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# THE MINUTE VOLUME OUTPUT AND THE WORK OF THE HEART IN HYPOTHYROIDISM<sup>1,2</sup>

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Close association between aberrations in the function of the thyroid gland and disturbances in the physiology of the cardiovascular system has long been recognized. Because of the frequent occurrence of evidences of heart disease in patients with thyrotoxicosis many investigators have studied the cardiac minute volume output and other aspects of the circulation in this disease. In hypothyroidism, on the other hand, evidences of disturbed cardiovascular function are less prominent, and the cardiac output accordingly has been less thoroughly investigated.

In 1925, Means (1) reported marked diminution in the cardiac minute volume output in two cases of myxedema studied by Field and Bock. Two years later, Mobitz (2) observed a decrease in the cardiac output in one patient with myxedema, and Kininmonth (3) likewise observed a decrease in two additional cases. The results of the two latter observers are difficult to interpret, since the validity of the original ethyl iodide method (4) which they utilized has been questioned. Several years later, Bansi (5) (6) using the methods of Douglas and Haldane (7) and of Grollman (8), also found decreased cardiac output in three patients with myxedema.

The recent work of Blumgart and his associates (9) (10) (11) in establishing the value of total ablation of the normal thyroid gland in the treatment of chronic intractable heart disease made it advisable to study the cardiac output and related aspects of the circulation in hypothyroidism induced by this operation.

## MATERIAL AND METHODS

Observations have been made on seven patients in whom hypothyroidism developed subsequent to total abla-

<sup>1</sup> This paper is number XVII of the series entitled "Therapeutic Effect of Total Ablation of Normal Thyroid on Congestive Failure and Angina Pectoris."

<sup>2</sup> This investigation was aided by a grant from the William W. Wellington Memorial Research Fund of Harvard University.

tion of the thyroid gland performed for the relief of angina pectoris. Studies were made in two, both before, and at different levels of basal metabolism after operation; in one other, observations were made at three different levels of metabolism after operation. Patients with no history of congestive failure were chosen for this study in order to avoid the complicating effect of cardiac decompensation on the output of the heart. Four subjects were males and three females; the ages varied from 53 to 65 years. Several patients showed slight elevation of the blood pressure; all presented slight to moderate sclerosis of the peripheral arteries. The hemoglobin in every instance was 90 per cent or above. Studies were made only in patients with hypothyroidism who could be trained to breathe as deeply and as rapidly as is necessary for measurement of the cardiac output by the acetylene method. The decreased oxygen consumption in hypothyroidism results in a diminution of the oxygen difference between the consecutive gas samples taken for analysis. This increases the percentage deviation from the average of repeated measurements of the cardiac output; it was not possible to obtain reliable results in several patients. As indicated in Table I, many patients were receiving small doses of desiccated thyroid gland (Armour) at the time of study.

All measurements were made in the postabsorptive state, under basal conditions with the patients in the semi-recumbent position, after a rest of one-half to one hour. The basal metabolic rate was first measured in duplicate with a Collins Benedict-Roth spirometer and calculated according to the Aub-DuBois normal standards. The arterial blood pressure was next measured using a mercury manometer with the standard cuff. The figures in the table represent the averages of several readings. The pulse rate was counted twice before each measurement of the cardiac output. Two measurements of the arteriovenous oxygen difference were made by the acetylene method of Grollman (8), three gas samples being taken for each measurement as suggested by Grollman, Friedman, Clark and Harrison (12). The values reported represent the average of two or more measurements made at the same level of basal metabolism. The maximum deviation from the average for any one value of the arteriovenous oxygen difference accepted as reliable was  $\pm 7.7$  per cent and the average deviation from the values reported was  $\pm 3.3$  per cent. The venous pressure was then measured by the direct method of Moritz and Tabora (13) and finally the velocity of blood flow was estimated, using as an index the "arm to tongue" circulation time measured with decholin (14).

The figures for the velocity of blood flow are the averages of two or more readings which did not differ from each other by more than two seconds. In three instances the velocity of blood flow was not measured on the same day as the cardiac output.

The cardiac minute volume output was calculated from the arteriovenous oxygen difference and the oxygen consumption, the latter being estimated from data obtained during the measurement of the basal metabolic rate. The work of the left ventricle was calculated using the formula of Evans and Matsuoka (15),  $W = QR + (wV^2/2g)$ ,<sup>3</sup> disregarding the velocity component  $wV^2/2g$  since it represents only 1 to 3 per cent of the total work.

### RESULTS

**Cardiac output and work.** The minute volume output of the heart was decreased in all 7 patients with hypothyroidism following total thyroidectomy (Table I, Figure 1). Measurements, made when the basal metabolic rates had decreased to between minus 25 and minus 37 per cent, re-

<sup>3</sup>  $W$  = work;  $Q$  = cardiac output per unit of time;  $R$  = arterial resistance (mean blood pressure  $\times 13.6$ );  $V$  = velocity of blood in aorta;  $w$  = weight of blood;  $g$  = acceleration due to gravity.

vealed cardiac indices (cardiac output in liters per square meter of body surface) of only 1.1 to 1.4 (Figure 1) as compared to the normal values of

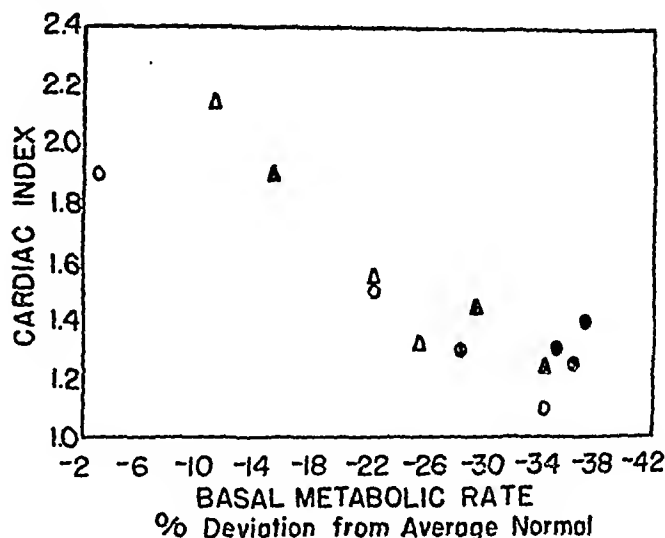


FIG. 1. RELATION BETWEEN THE BASAL METABOLIC RATE AND THE CARDIAC INDEX.

The dots indicate measurements made on various patients at a single low level of metabolism. Measurements at various levels of metabolism in three patients are indicated by the circles, and open and closed triangles.

TABLE I  
*Cardiac output and related observations in hypothyroidism*

Case	Age	Height	Weight	B. M. R.: deviation from normal	Oxygen consumption	Arteriovenous oxygen difference	Cardiac minute volume output	Cardiac index	Pulse rate	Cardiac output per beat	Blood velocity	Venous pressure	Arterial pressure, systolic	Arterial pressure, diastolic	Vital capacity	Work per minute	Work per beat	Remarks
	years	inches	pounds	per cent	cc. per minute	volumes per cent	liters		per minute	cc.	seconds	cm. H <sub>2</sub> O	mm. Hg	mm. Hg	cc.	kilogram- meters	gram- meters	
Cases studied after total thyroidectomy																		
1. M. P. . .	53	62½	137	-37	133	5.85	2.3	1.4	55	41	32		130	86	3600	3.3	61	4 months after operation
2. R. S. . .	58	59½	139	-28	138	6.7	2.1	1.3	65	32			186	110	1500	4.1	64	11 months after operation
3. T. C. . .	65	70	178	-36	160	6.35	2.5	1.2	46	55	21	4.1	138	90	3750	3.9	85	Thyroid 1/10 grain daily
4. G. O. . .	65	69½	194	-35	167	6.5	2.6	1.3	58	44	16	6.9	120	76	2350	3.4	59	10½ months after operation
																		Thyroid 1/10 grain daily
																		14 months after operation
																		Thyroid 1/10 grain daily
Cases studied before and after total thyroidectomy																		
5. R. B. . .	57	60½	164	-11	184	5.00	3.7	2.1	68	54	18	6.0	160	100	1900	6.5	96	Before operation
			162	-25	156	6.67	2.3	1.4	60	39	23		140	82		3.4	57	6 weeks after operation
			165	-22	163	6.20	2.6	1.5	69	38			140	80	1700	3.9	57	4 months after operation
6. M. S. . .	57	58	107	-3	163	5.95	2.7	2.0	73	38	17.5	8.0	160	90	1550	4.7	64	Thyroid 1/5 grain daily
			113	-22	135	6.23	2.2	1.5	74	29	21	7.7	164	102	1550	3.9	53	Before operation
																		4 months after operation
			124	-34	118	6.93	1.7	1.1	63	27	27	8.9	150	94	1500	2.8	45	No thyroid for 5 weeks
																		No thyroid for 7 weeks
Case studied at various levels of basal metabolic rate after operation																		
7. S. F. . . .	53	64.5	163	-34	154	6.95	2.2	1.2	56	40	31		110	90	2100	3.0	54	4 months after operation
			153	-15	193	5.85	3.3	1.9	79	42	22		116	85	2700	4.5	58	5 months after operation
																		Thyroid 1 grain, t.i.d.
			163	-29	165	6.35	2.6	1.4	76	34	37	3.1	104	76	2500	3.2	42	9 months after operation
																		Thyroid ½ grain every 3 days

$2.2 \pm 0.3$  (16). The work of the left ventricle was correspondingly diminished.

The findings in Cases 5 and 6 studied both before and after operation, and in Case 7 studied at different levels of metabolism after operation indicate that as the basal metabolic rate falls, the minute volume output of the heart decreases progressively more rapidly than the oxygen consumption.

*Arteriovenous oxygen difference.* An increase of arteriovenous oxygen difference became evident at low levels of metabolism (Table I, Figure 2). The disproportionate decrease in the cardiac

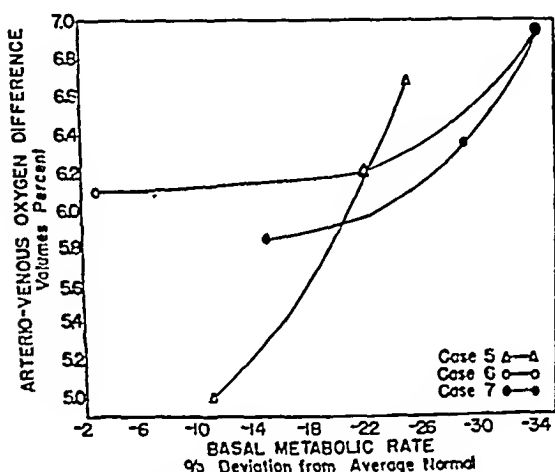


FIG. 2. RELATION BETWEEN ARTERIOVENOUS OXYGEN DIFFERENCE AND THE BASAL METABOLIC RATE IN THREE PATIENTS STUDIED AT VARIOUS LEVELS OF BASAL METABOLISM.

output noted above is associated with this progressive increase in arteriovenous oxygen difference (Cases 5, 6, and 7, Table I).<sup>4</sup>

*Velocity of blood flow.* The velocity of blood flow was usually decreased when hypothyroidism had developed (Table I). In Cases 3 and 4 the velocity of blood flow was not appreciably less than normal although the cardiac output was strikingly decreased. In some instances, therefore, the velocity of blood flow did not reflect accurately the work of the heart.

*Venous pressure, arterial pressure, and vital capacity.* The venous and arterial blood pressure

and vital capacity showed no significant changes after total thyroidectomy (Table I).

## DISCUSSION

Starr, Collins, and Wood in 1933 (17) demonstrated a high coefficient of correlation between the cardiac output and oxygen consumption in normal persons under basal conditions. The results of the present study in patients with hypothyroidism indicate, however, that as the basal metabolic rate falls, the minute volume output of the heart decreases progressively more rapidly than the oxygen consumption. This disproportionate decrease in cardiac output in hypothyroidism is accompanied by an increase in the arteriovenous oxygen difference and is in harmony with the observations of Field and Bock in two cases of spontaneous myxedema (1).

The increased arteriovenous oxygen difference found in hypothyroidism is not to be regarded as evidence of heart failure due to "myxedema heart." An increase has in fact been observed after total thyroidectomy in patients with congestive heart failure when the signs of cardiac decompensation were disappearing (unpublished data). The fact that the venous pressure was not increased in the above studied patients with marked hypothyroidism is additional evidence that the circulatory changes were not due to cardiac decompensation. Estimation of the size of the heart on x-ray examination before and after operation, furthermore, revealed enlargement after hypothyroidism had developed in only three of the seven patients of this series. The factor responsible for the increased arteriovenous difference has not been ascertained. Further work in this direction is now in progress.

Calculations from the data obtained in this study show that the work of the left ventricle is greatly diminished at low levels of metabolism following total thyroidectomy. This is in harmony with the concept advanced by Blumgart et al. (9, 10, 11) that the relief obtained in congestive failure and angina pectoris after thyroidectomy is due to lowered demands upon the heart in the hypothyroid state. It is significant that the patients studied experienced relief of their angina pectoris when the work of the heart decreased. Conversely, patients with spontaneous myxedema may

<sup>4</sup> Drs. T. R. Harrison and Harold J. Stewart have each studied one case after total thyroidectomy and have also observed this change in the arteriovenous oxygen difference. (Personal communications.)

develop angina pectoris when treated with thyroid (18).

In hypothyroidism the marked decrease in cardiac output with its associated increase in arteriovenous oxygen difference results in a disproportionately greater decrease in left ventricular work than in basal metabolism. At very low levels of metabolism the rest afforded the heart becomes considerably greater than that which might be expected from the decrease in basal metabolic rate alone.

### CONCLUSIONS

1. The minute volume output and the work of the heart are greatly diminished in hypothyroidism following total ablation of the normal thyroid gland.

2. The cardiac output decreases progressively more rapidly than the oxygen consumption as the basal metabolic rate falls in hypothyroidism. This disproportionate decrease in cardiac output is accompanied by a progressive increase in the arteriovenous difference.

3. In most instances the velocity of blood flow was decreased when the cardiac output was low. In some instances, however, the velocity of blood flow did not reflect accurately the work of the heart.

4. The venous pressure, arterial pressure, and vital capacity were not significantly altered after total thyroidectomy in the patients of this series.

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# IMMUNITY IN DIABETES. II. RELATIVE IMPORTANCE OF NUTRITIONAL STATE AND OF BLOOD SUGAR LEVEL IN INFLUENCING DEVELOPMENT OF THE AGGLUTININ AFTER TYPHOID VACCINE

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In a previous study it was shown that diabetic patients form antibodies in smaller amounts than non-diabetic controls (1). In the work demonstrating this, typhoid vaccine was used as the antigen and the agglutinin response was measured by Dreyer's macroscopic method. It was further shown that those patients who were in a poor clinical condition formed antibody in less amount than those whose condition was satisfactory.

The diabetic in poor condition presents a picture of hyperglycemia with undernourishment and loss of weight. With this there is an alteration in the amount and distribution of the body glycogen. It seemed, therefore, that a study of antibody formation in animals which, while not diabetic, were on the one hand undernourished, or on the other hand hyperglycemic, might aid in determining the significance of these factors in the decreased antibody formation of the diabetic. ✓

## METHODS

Normal adult rabbits, weighing about three kilograms, were used for these experiments. The antigen selected was standard *B. typhosus* vaccine. Two doses, of 0.1 cc. each were given intravenously one week apart, except in Experiment Number 2, in which 0.4 cc. was given. The intravenous method of inoculation was used in order to avoid variations in absorption. Blood for agglutination tests was taken before each experiment and one week after the second dose of vaccine. Dreyer's macroscopic method was used for measuring the agglutinative titre of the serum in which agglutination occurred. In none of the rabbits did the blood show any agglutinative titre before the vaccine was given. Undernourishment was induced by feeding an insufficient diet, and hyperglycemia by the subcutaneous injection of epinephrine. Blood sugar determinations were done by the Benedict method. —

In each of the under-feeding experiments the

rabbits were weighed and divided into two groups. In the control group they were fed throughout the experiment the standard diet of the laboratory with the addition of carrots, beets, and oats amounting in all to double the basal caloric requirement. In the other group the rabbits were fed, throughout the experiment, the same variety of food reduced in amount, however, so that they received one half of the basal caloric requirement. After one week on these diets the animals were weighed and given the first dose of *B. typhosus* vaccine. One week later the same dose of vaccine was repeated. One week after this injection, blood was taken for measurement of the agglutinative titre. During this time the under-fed rabbits lost an average of 477 grams in weight. The control rabbits, on the other hand, maintained their previous weight, or in some cases increased slightly. Determinations of the concentration of glycogen in liver tissue were made as a further check on the state of nourishment. Liver tissue was removed under nembutal anesthesia from some animals in each group. The method used is a modification of the method of Cori, using cold KOH, as described by Evans (2). The under-fed rabbits had between 0.7 per cent and 2.0 per cent of glycogen in the liver, while the control animals had between 4.2 and 9.2 per cent. The results of these experiments are given in Table I. There was a marked difference in the appearance of the well and of the poorly fed rabbits after each dose of vaccine. The former showed no symptoms following the vaccine, even continuing to eat normally.— The latter, however, were definitely ill, and in all except one experiment one or more of these rabbits died within twenty-four hours after an injection. In Experiments Number 2, 5 and 6 there was a definitely smaller amount of antibody formed by the poorly nourished rabbits. In Experiment Number 4, while the difference is not so clearly evident, there was

TABLE I

*Effect of restricted diet compared with that of liberal diet on the agglutinative titre developed after intravenous typhoid vaccine*

Experiment number	Rabbits fed low caloric diet: one-half basal requirement		Rabbits fed high caloric diet: twice the basal requirement	
	Rabbit number	Agglutinative titre	Rabbit number	Agglutinative titre
2	232	died	248	1/10240
	244	1/160	249	1/5120
	245	died	250	1/5120
	246	1/2560		
	247	died		
4	269	1/320	252	1/1280
	267	1/40	251	1/5120
	266	1/640	248A	1/5120
	262	1/160	264	1/160
	250B	1/1280	253	1/5120
	249A	1/2560	265	1/320
	268	1/320	256	1/5120
5	186A	died	182A	1/10240
	191A	1/5120	180A	1/10240
	187A	died		
	183A	1/2560		
6	188B	1/1280	248B	1/20480
	273B	1/2560	275B	1/10240
	272B	died		
		Glycogen content of liver		Glycogen content of liver
		per cent		per cent
2	232	0.7	248	9.1
	244	1.7	249	8.4
	245	0.9	250	4.2
	246	2.0		
	247	1.1		
6	188B	0.7	248B	9.2
	273B	1.6	256	8.8

TABLE II

*Effect of regular diet compared with that of liberal diet reinforced with glucose on the agglutinative titre developed after intravenous typhoid vaccine.*

*Experiment Number 1*

Rabbits without glucose and with regular diet		Rabbits with glucose and high caloric diet	
Rabbit number	Agglutinative titre	Rabbit number	Agglutinative titre
183	1/20480	180	1/40960
184	died	181	died
185	died	182	1/81920
188	1/20480	186	1/40960
189	1/20480	187	1/81920

glutination test was taken one week after the second dose. During the experiment the rabbits which received normal salt solution were given the regular laboratory diet, while those which had glucose received such food as carrots and oats in larger quantity. One rabbit in the latter group died following the vaccine while two in the control group died. In this experiment also the animals with the greater caloric intake consistently formed more agglutinin than did the others.

It appears from these experiments that animals which are well fed are better able to form antibody than those which have a lower caloric intake.

In the experiments on hyperglycemia the rabbits were fed a full diet, the same as that given to the control rabbits in the first group of experiments described. In each experiment they were divided into two groups, one receiving epinephrine injections, the other acting as controls. Both groups were given *B. typhosus* vaccine, and blood was taken for agglutination as in the former experiments. Two injections of 1.0 cc. each of 1-1000 solution of epinephrine were given to the epinephrine group subcutaneously daily at 9 a.m. and 4 p.m. for seven days before the first dose of vaccine and continuously throughout the experiment. Two rabbits out of thirteen which received epinephrine died before any vaccine was given them. The vaccine was given one hour after the 9 o'clock epinephrine injection so that the blood sugar was between 240 and 410 mgm. per 100 ml. of blood at the time. Blood for sugar determination was taken at intervals up to five hours after the injections of epinephrine and was found to contain from 150 to 253 mgm. of

a probably significant difference between the results in the two groups. The average of the logarithm of the titre for the undernourished rabbits in Experiment Number 4 is  $2.6 \pm 0.22$  and that for the well nourished is  $3.2 \pm 0.19$  giving a difference of  $0.6 \pm 0.29$ .

In Table II are shown the results of a slightly different experiment. Here one group of rabbits received six intravenous injections of 8 cc. of 25 per cent solution of glucose at hourly intervals immediately before the vaccine was given. The control group received normal salt solution in place of the glucose solution. One cc. of vaccine was given intravenously, and the same procedure, administration of glucose followed by vaccine, was repeated one week later. Blood for ag-

sugar per 100 ml. of blood. There was considerable variation in the effect of the epinephrine on the blood sugar of different animals, but on the whole it appeared that hyperglycemia was present for from seven to fourteen hours in each day.

The results of these experiments are shown in Table III. There was no significant difference

TABLE III

*Effect of hyperglycemia induced by repeated subcutaneous injections of epinephrine on the agglutinative titre developed after intravenous typhoid vaccine*

Experiment number	Rabbits with hyperglycemia		Rabbits with normal blood sugar	
	Rabbit number	Agglutinative titre	Rabbit number	Agglutinative titre
3	252C	1/20480	251C	1/20480
	253C	1/40960	256C	1/40960
6	250B	1/10240	248B	1/20480
			275B	1/10240
7	294	1/20480	291	1/20480
	20	1/5120	22	1/40960
	23	1/5120		
8	25	died	26	1/2560
	27	1/10240	31	1/10240
	28	1/5120		
	296	1/10240		
	298	1/320		

between the agglutinative titres of the two groups. It would seem from these experiments, therefore, that hyperglycemia maintained by repeated injection of epinephrine has little or no effect on the amount of antibodies appearing in the blood.

In a previous investigation we determined the ability of the diabetic to form antibodies as compared with that of the non-diabetic, by making agglutination tests on 41 diabetics and 39 non-diabetics following typhoid vaccine given according to the usual method (1). A reexamination of our records of these experiments shows that from 2 to 4 blood sugar determinations were made on each patient during the period of the inoculations. Eighteen patients developed an agglutinative titre of 1/5,120 to 1/20,480; 18 other patients agglutinated the antigen in dilutions of only 1/80 to 1/1280. In the high titre group the blood sugar determinations varied from 100 to 360 mgm. per 100 ml. with an average of  $204 \pm 22$  mgm. In

the low titre group the blood sugar varied between 135 and 348 mgm. per ml. with an average of  $225 \pm 19$  mgm. It is evident that those patients who developed a low and those who developed a high agglutinative titre did not essentially differ in their blood sugar concentration.

In the same study (1) the bactericidal power was measured by adding the blood of diabetics and of non-diabetics to dilutions of from 1/10 to 1/1,000,000 of broth cultures of *B. coli*, *Pneumococcus*, *Staphylococcus aureus*, *Streptococcus hemolyticus* and *Streptococcus viridans*. The killing power of the bloods of patients and controls was compared (see (1) Table II). The results from patients with blood sugar under 200 mgm. and over 200 mgm. per 100 ml. of blood were grouped separately. It was evident that there was no significant difference in the bactericidal power of the two groups. These findings are consistent with those reported in this study. ✓

#### DISCUSSION

Antibody formation may probably be taken as a measure of a part of the defense of the body against infection, and a deficient antibody formation as evidence of disordered cellular metabolism. Two features are common to diabetic patients and to the under-fed rabbits of these experiments; namely, a low caloric intake and a lowered glycogen concentration in certain tissues. We cannot assert that the impairment in cellular metabolism which leads to the deficient antibody formation is the same in these under-fed rabbits and in the diabetic. However, inasmuch as hyperglycemia *per se* had no significant influence on the antibody formation, whereas the state of nutrition apparently did, it is suggested that the susceptibility to infection on the part of the diabetic is due to a disordered cellular nutrition closely associated with the diminution of cellular glycogen reserve.

#### CONCLUSIONS

Under-fed rabbits with depleted liver glycogen developed definitely lower agglutinative titre after typhoid vaccine than did well-fed controls with higher liver glycogen. Hyperglycemia maintained by repeated doses of epinephrine had no significant influence on the agglutinative titre developed in rabbits after typhoid vaccine. These



results are discussed in relation to observations on diabetic patients that no correlation was observed in diabetics between fasting blood sugar concentration and either the agglutinative titre developed after typhoid vaccine or the bactericidal power of the diabetic blood.

It is suggested that the state of cellular nutrition is more important than the level of blood sugar in determining the antibody response.

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(From The Department of Physiology, New York University College of Medicine,  
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absorption and, more particularly, of a possible passive diffusion of substances of low molecular weight across the tubules (especially at high U/P clearance ratios) are not excluded by the above evidence as satisfactorily as might be. Jolliffe, Shannon and Smith (1932a) were led to believe that there was neither active nor passive reabsorption of xylose, sucrose and raffinose by the identity of the simultaneous clearances of these substances in the normal animal, and the relative constancy of these clearances with respect to the urea clearances before and after phlorizin.

It was principally with this question of possible reabsorption in mind, and prompted by the above view regarding the probable non-secretion of carbohydrates, that we extended our investigations to the substance reported on here, namely, inulin. A preliminary report on the excretion of inulin in the dog has been made by Richards, Westfall and Bott (1934). They find that this substance is not excreted by the aglomerular kidney of *Opsanus*, whereas when injected intravenously into the dog it is excreted rapidly and completely, and in approximately the same ratio to its concentration in the plasma as is creatinine. They observe that the xylose clearance is lower than the simultaneous inulin clearance. Professor Richards, in his recent Harvey Lecture (January 17, 1935), reported further that inulin injected into the frog is excreted in the glomerular urine at the same concentration as it is present in the plasma; that it appears to be completely filterable; and that it is not excreted by the frog's kidney when supplied only to the tubular and not to the glomerular circulation. A report on the excretion of inulin in the dogfish has been made by Shannon (1934b), who has found that the xylose clearance is consistently lower than the simultaneous inulin clearance (averaging 78 per cent of the latter) in the normal animal, and equals the latter in the phlorizinized animal.

Inulin is a polysaccharide, widely distributed as a reserve material in many plants, which on hydrolysis yields fructose. The molecular weight of the unhydrated compound is variously given from 972 to 4860 (Pringsheim (1932)) and is possibly nearer to the latter figure (Irvine and Montgomery (1933); Drew and Haworth (1928); Haworth, Hirst and Percival (1932)),

although the available evidence indicates that scission occurs very readily at elevated temperatures.

The inulin used in these experiments, obtained from Pfanstiehl Chemical Company, is a white and presumably pure preparation having a negligible reducing power. It dissolves in 0.6 per cent NaCl at 85° C. to the extent of 20 per cent by weight and remains in solution long enough when cooled to 40° C. to permit intravenous infusion. It is not bound by plasma protein and is diffusible through a collodion membrane (Shannon (1934b)).

Experiments on the dog will be published elsewhere, the present paper being concerned with the simultaneous clearances of inulin, xylose, urea and glucose in normal and phlorizinized man.

#### EXPERIMENTAL PROCEDURE

Prior to this work inulin had not been administered parenterally to humans, and for this reason, after extensive experiments on the dog, an intravenous infusion of 160 grams was taken by one of the investigators (J. A. S.). There were no objective or subjective disturbances, nor have any ill effects been observed to follow intravenous infusion into a number of individuals since that time.<sup>2</sup> The observations reported here were made on seven volunteer convalescent patients from the wards of the Medical Service of the Third (New York University) Medical Division of Bellevue Hospital, with apparently normal renal function. They were chosen on the basis of history, ability to concentrate urine to a specific gravity above 1.030, absence of albumin and a normal urinary sediment.

The inulin was dissolved in sterile 0.6 per cent NaCl (20 grams to each 100 cc.) by heating to a temperature of 85° C. The resulting solution was invariably clear and had a faint yellow tint. On cooling to 40° C. recrystallization does not take place for some time, but the solution for injection was prepared just prior to use to prevent the necessity of a second heating. One hundred grams of inulin as given here yield a blood concentration starting at 300 to 400 mgm. per cent, and falling to 100 mgm. per cent during the next 1.5 to 3 hours.

Schering-Kahlbaum's phlorizin was recrystallized according to the method of Deuel and Chambers (1925). This was weighed out in advance and just before use suspended in enough 2.5 per cent NaHCO<sub>3</sub> to make an 8 per cent solution. This suspension was gently heated until solution was effected and, after being cooled to 38° C., administered at once by slow intravenous injection.

<sup>2</sup> After 42 instances with no reaction, a severe reaction was observed with a new lot of inulin. The difficulty has not been ascertained but is under investigation at the time of reading proof.

The full routine of preparation and observation follows (in those instances in which all substances were not present there was little alteration in the procedure save for the omission of the xylose, creatinine or phlorizin, as the case may have been):

The subject was allowed no breakfast. During the two hours preceding the beginning of observations 2000 cc. of water were given by mouth. At zero hour 100 grams of xylose and 10 to 15 grams of creatinine were taken in 300 to 400 cc. of chilled water. The inulin infusion was started at 30 minutes and so regulated as to be completed in an additional 30 minutes. A catheter was inserted in the urethra at 80 minutes, and after a preliminary wash-out of the bladder with warm sterile water the first period of urine collection was started at 90 minutes. All subsequent urine collections were made by catheterization, with every care to empty the bladder completely.

The periods of urine collection varied from 10 to 20 minutes in duration and in number from 3 to 12, depending upon the observations desired. When these observations were to include phlorization, this was done after 3 control periods and followed by a 10 minute washout period after the injection of the phlorizin, and then 3 more periods of observation. Samples of blood were taken as close to the middle of the periods of urine collection as possible and interpolated exactly to that point, one blood sample being taken for each urine collection. In the long experiments 200 cc. of water each hour were sufficient to insure a high rate of urine flow, the ingestion of the water causing no apparent change in clearance.

#### CHEMICAL METHODS

Coagulation was prevented in the blood by the use of heparin. The blood was centrifuged as soon as drawn and precipitated immediately. Urine samples were diluted at once to the expected U/P ratio and precipitated at the termination of the experiment. Urea was determined by Van Slyke's (1927) manometric method and creatinine by the method used by Shannon, Jolliffe and Smith (1932). The ferric sulfate precipitation permits both creatinine and sugars to be done on the same filtrate. All analyses were done in duplicate, and if these did not agree a third set was run. The figures cited are an average of the duplicates, or of the closest two out of three determinations. Precipitation of plasma and diluted urine was effected in 1:5 or 1:10 dilution, as seemed advisable for accurate analysis. The method of Steiner, Urban and West (1932) was used, utilizing ferric sulfate followed by  $\text{BaCO}_3$ . The barium in solution was subsequently precipitated by addition of 1 drop of saturated  $\text{Na}_2\text{SO}_4$  to each 15 cc. of filtrate.

All sugar analyses were made by the Shaffer-Somogyi (1933) method, using Reagent Number 50 containing 5 grams of KI per liter. A variable quantity of filtrate was taken (2 to 5 cc.) depending upon the amount of sugar present, and water added to total 5 cc., to which were added 5 cc. of the copper reagent. Whenever possible enough filtrate was used to make the titration

small, but not less than 1 cc. Two reagents were used, one having an iodate content equivalent to 17.5 cc. of 0.005 N thiosulfate, the other to 10.0 cc. of 0.005 N thiosulfate. Blanks were run with each set of sugar determinations, and standards were included every day. The correspondence between duplicates was excellent. Xylose was determined as reducing power after destruction of glucose by yeast. Glucose was removed from both plasma and urine filtrates by treatment with yeast, whether xylose was present or not. The supernatant fluid after the yeast is centrifuged out must be absolutely starch free, of course, for inulin determination. We have recently found Fleischmann's starch-free yeast very satisfactory. It can be procured in pound lots and keeps at least two weeks on ice. The tinfoil yeast contains as a binder, starch, which is very difficult to remove by washing. Inulin was hydrolyzed in the sugar tubes by adding 0.5 cc. of  $\text{N H}_2\text{SO}_4$  to 5.0 cc. of the iron filtrate, heating in a water bath for 15 minutes, cooling and neutralizing exactly to phenolphthalein with  $\text{N KOH}$ . Hydrolyzed in this manner, our sample of inulin has a glucose equivalent of 94 per cent; this reducing power is the same in aqueous solutions hydrolyzed directly, or when inulin is added to plasma or urine and precipitated as above, as evidenced by a series of 14 sets of simultaneous recoveries from water, plasma and urine at concentrations ranging from 50 to 500 mgm. per cent of inulin, both with and without the presence of known amounts of xylose. The maximum variation of plasma and urine from the aqueous solution was 2 per cent. Inulin in concentrations equivalent to 500 mgm. per cent in the plasma does not modify the color produced in the Jaffe reaction for creatinine if read within 15 minutes. The inulin concentration has been taken to be the difference between the total reducing power expressed as glucose before and after hydrolysis when xylose is present, or after hydrolysis alone in the absence of xylose. The latter procedure is justified since the saccharoid content of blood, using the above method, is of the order of magnitude of 1 to 2 mgm. per cent. Phlorizin has negligible reducing power and is not appreciably hydrolyzed by the treatment accorded inulin. Creatinine has a reducing power of 31 per cent of its weight in glucose equivalent by this method, either alone or in the presence of sugars, and appropriate corrections must be made. In the sugar determinations, corresponding plasmas and urines were handled simultaneously. All pipettes were specially constructed and standardized to blow out, and were cleaned in cleaning solution after each use. The smallest pipette used was 2.0 cc. We cannot place too much emphasis upon the need for precautions of this type in all sugar methods.

#### RESULTS

After intravenous injection, inulin is apparently quantitatively excreted in the urine in a period of 24 hours. Data on this point are given in the last column of Table I. Our data are complicated by



TABLE I

*Summary of observations on normal men*

Subject	Surface area	Number of observation periods	Average inulin clearance	Average urea inulin	Average xylose inulin	Inulin given	Inulin excreted in 24 hours
	<i>square meters</i>					<i>grams</i>	<i>per cent</i>
J. A. S...	2.10	2	142	0.65	0.80	160	92
M. C....	2.08	4	129	0.59	0.82		
R. S....	1.75	6	141		0.71		
B. R....	1.67	4	93		0.81		
		3	84	0.67	0.81	100	95
W. F....	1.69	6	82		0.78	100	97
		9	72		0.79	100	98
		3	76	0.65	0.80	100	103
J. C....	1.63	9	110		0.76		
S. C....	1.79	3	123	0.62	0.73		
		3	128		0.79		
Average .				0.635	0.78		97

the fact that facilities were not available at the time of these observations to insure the complete injection of an exact quantity of inulin; there was always a slight loss at the beginning of the injection

in clearing the infusion apparatus of air, and again at the termination of the injection when some of the solution is necessarily lost through wetting the gravity bottle and the small quantities remaining in the connecting tube. This loss was not taken into account in our figures, but a 2 per cent correction would probably suffice. Our 24 hour urines show, however, that the recovery of inulin is essentially complete. In two instances not recorded in Table I, we recovered 95 and 98 per cent.

It could be demonstrated that the clearance of inulin is independent of plasma level from observations designed to answer other questions, but in certain instances (Figure 1) we have examined this point particularly. The data in Figure 1 show that the renal excretion of inulin is directly proportional to the plasma level (i.e., the clearance is constant) over a fourfold change of the latter. This question has not been examined at plasma levels below 50 mgm. per cent, but that this linear relationship obtains throughout the range from 0

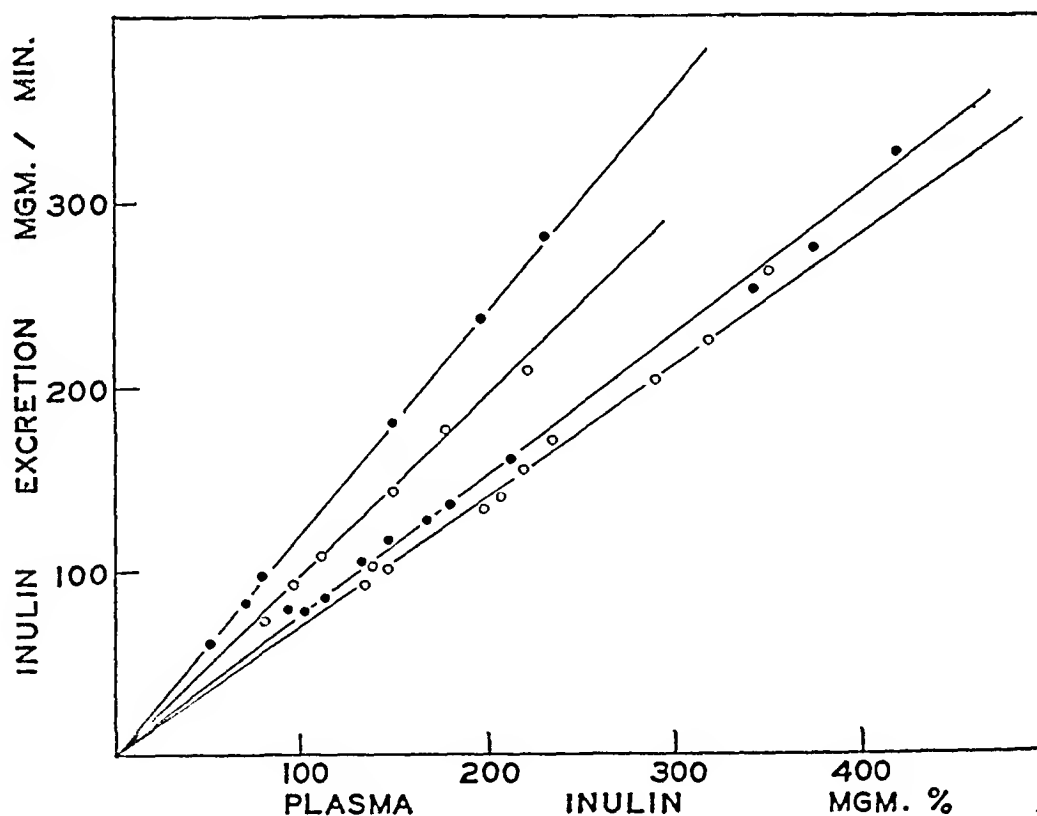


FIG. 1. DATA ON FOUR EXPERIMENTS AT VARIOUS CLEARANCE LEVELS SHOWING THAT THE RATE OF INULIN EXCRETION IS PROPORTIONAL TO THE PLASMA CONCENTRATION.

Each point is a single clearance period. The solid dots and open circles indicate alternate experiments each of which is indicated by a straight line.

to 50 mgm. per cent in the plasma is indicated by the fact that the straight lines generated at higher plasma levels extrapolate to the intersection of the coordinates. (The rate of fall of the plasma concentration of inulin is, after the first 40 minutes from the end of the infusion, exponentially related to time. This is a necessary consequence when the rate of removal from the blood is a linear function of the plasma level, but it reveals nothing about the mechanism or mechanisms involved in this process.)

Table I shows that the clearance of xylose in normal man is less than the clearance of inulin by an average of 22 per cent, while the creatinine clearance in our observations bears essentially the same relationship to xylose as has been described by Jolliffe and Chasis (1933). Although the creatinine data must be deferred to a subsequent paper, we may note here that at plasma levels of creatinine from 5 to 10 mgm. per cent the creatinine clearance exceeds the inulin clearance by 30 to 45 per cent. This corresponds to a creatinine/xylose clearance ratio of 1.67 to 1.86, as compared to the average figure of Jolliffe and Chasis of 1.73. Our urea/xylose clearance ratios are higher than were observed by these authors, but our series is smaller.

The relationship between xylose and inulin clearances in normal man require special comment. It will appear from Table I that this ratio varies somewhat from one subject to another, although in a single individual the variation is slight (see Figure 2). The data upon which Figure 2 is based were obtained from one man (W. F.) over a period of 6 weeks. The mean xylose/inulin clearance ratio in this individual is 0.78, with a maximum variation of  $\pm 0.04$ . It can be seen from Figure 2 that this ratio is not dependent upon the U/P ratio over the range examined (6 to 30), but it must be noted that the highest U/P ratio (30) was obtained at a urine flow of above 2 cc. per minute. No attempt has been made to examine this ratio over lower ranges of urine flow.

Chasis, Jolliffe and Smith (1933) found that less than 65 mgm. of phlorizin were required to bring the glucose clearance up to the xylose clearance, but for reasons to be discussed in a subsequent paper, we increased this dose to 100 mgm.

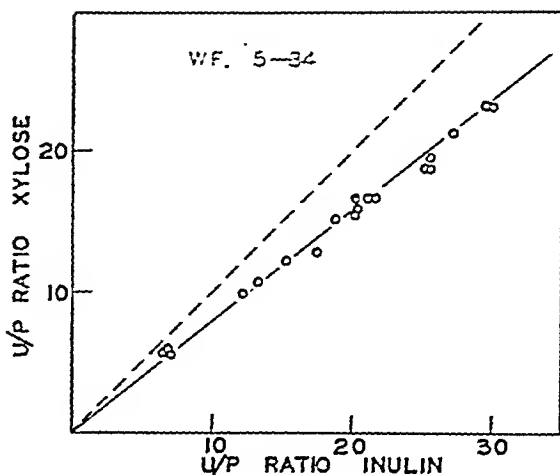


FIG. 2. DATA ON ONE INDIVIDUAL SHOWING THAT THE XYLOSE U/P RATIO (AND THEREFORE THE CLEARANCE) IS LESS THAN THE SIMULTANEOUS INULIN CLEARANCE.

The average xylose/inulin clearance ratio in this individual was 0.78, and showed little variation over a period of six weeks. The broken line indicates a slope of 1.00. Each point is based on a single pair of simultaneous inulin and xylose clearances.

per kilogram. (This dose was taken by J. A. S. in a preliminary trial.) Detailed results of our observations before and after phlorizin are given for a single individual in Table II, and a summary of observations on five individuals is given in Table III. When phlorizin is administered in doses of 100 mgm. per kilogram the glucose/inulin clearance ratio is raised from 0. to 0.91, and the xylose/inulin clearance ratio from an average of 0.79 to 0.89. The urea/inulin clearance ratio is not changed (0.62 before, and 0.62 after phlorizin). As was previously shown by Chasis, Jolliffe and Smith (1933) the glucose clearance rises to, but does not significantly exceed, the xylose clearance. The above authors found the same urea/xylose clearance ratio before and after phlorizin, whereas we find a slight decrease (from 0.78 to 0.70). It should be noted, however, that the clearances of all the substances fall to a variable degree after the administration of phlorizin, and in view of this fact it is difficult to evaluate the significance of the change in the urea/xylose clearance ratio.

In Table IV we have included data to show that the intravenous infusion of inulin to man has no effect upon the xylose or urea clearances. This

TABLE II

*Observations on W. F. before and after phlorizin. May 20, 1934*

Period number	Duration	Urine flow	Plasma level				Clearance				Clearance ratios			
			Urea	Xylose	Inulin	Glucose	Urea	Xylose	Inulin	Glucose	$\frac{\text{Urea}}{\text{Inulin}}$	$\frac{\text{Xylose}}{\text{Inulin}}$	$\frac{\text{Glucose}}{\text{Inulin}}$	$\frac{\text{Glucose}}{\text{Xylose}}$
	minutes	cc. per minute	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	cc. per minute	cc. per minute	cc. per minute	cc. per minute				
1	10	10.4	30.3	74.5	337		46.8	57.8	73.3		0.64	0.79		
2	11	11.3	29.9	76.6	297		50.3	63.8	77.6		0.65	0.82		
3	12	11.1	29.6	81.7	274		51.8	61.3	76.6		0.67	0.80		
4	11	4.5	29.3	86.2	214	103	36.4	53.6	57.1	51.3	0.64	0.94	0.90	0.96
5	11	4.2	29.0	87.3	199	102	35.3	50.0	56.7	50.8	0.62	0.88	0.90	1.02
6	10	4.1	28.7	85.5	187	100	35.1	50.9	55.0	49.0	0.64	0.92	0.89	0.96

TABLE III

*Summary of experiments on phlorizinized man*

Before phlorizin					After phlorizin					
Subject	Number of periods	Average inulin clearance	Average clearance ratios		Number of periods	Per cent change in inulin clearance	Average ratios			
			$\frac{\text{Urea}}{\text{Inulin}}$	$\frac{\text{Xylose}}{\text{Inulin}}$			$\frac{\text{Urea}}{\text{Inulin}}$	$\frac{\text{Xylose}}{\text{Inulin}}$	$\frac{\text{Glucose}}{\text{Inulin}}$	$\frac{\text{Glucose}}{\text{Xylose}}$
J. A. S. ....	2	142	0.65	0.80	3	- 4.0	0.63	0.90	0.97	1.08
B. R. ....	3	85	0.67	0.82	3	-17.0	0.68	0.89	0.89	1.00
					4			0.88	0.91	1.02
W. F. ....	3	76	0.65	0.80	3	-32.0	0.63	0.91	0.90	0.99
J. C. ....	3	112			3	-11.0		0.92		
S. C. ....	3	123	0.51	0.73	3	-45.0	0.53	0.86	0.83	0.94
Average. ....			0.62	0.79		-20.6	0.62	0.89	0.91	1.01

TABLE IV

*Absence of effect of inulin infusion upon other clearances*

Period number	Time from zero hour	Urine flow	Plasma level			Clearances			Clearance ratios		
			Urea	Xylose	Inulin	Urea	Xylose	Inulin	$\frac{\text{Urea}}{\text{Xylose}}$	$\frac{\text{Urea}}{\text{Inulin}}$	$\frac{\text{Xylose}}{\text{Inulin}}$
	minutes	cc. per minute	mgm. per cent	mgm. per cent	mgm. per cent	cc. per minute	cc. per minute	cc. per minute			
1	132										
2	149	9.4	29.7	73.7		50.9	66.5		0.76		
	159	7.5	29.8	81.5		54.6	71.1		0.77		
Inulin infusion (see note below).											
3	338										
4	350	6.5	27.4	122.7	335	57.4	70.2	82.6	0.82	0.70	0.85
5	404	6.0	27.5	119.3	242	54.3	69.1	82.9	0.80	0.66	0.83
	419	5.6	27.6	122.3	252	56.0	69.0	86.9	0.81	0.64	0.79

Note: 100 grams inulin in 500 cc. of 0.6 per cent saline were given at the termination of Period 2 (from 210 to 240 minutes). At 250 minutes 25 grams xylose were given by mouth. At 333 minutes the bladder was washed out and the third period started at 338 minutes.

carbohydrate appears to be just as physiologically inert as are xylose and sucrose (Jolliffe, Shannon and Smith (1932b)).

#### DISCUSSION

No doubt the most noteworthy of these observations is the fact that the inulin clearance exceeds the simultaneous xylose clearance in normal man. In this we confirm the findings of Richards, Westfall and Bott (1934) in the dog. This disparity is open to three interpretations (see also Shannon (1934b)):

(a) The xylose clearance constitutes a measure of glomerular filtration, and inulin is in part excreted by the process of tubular secretion; (b) the inulin clearance is a measure of glomerular filtration and xylose is reabsorbed; (c) the level of glomerular filtration is above that of the inulin clearance.

The possibility that the level of glomerular filtration lies below the level of the xylose clearance (i.e., that xylose is secreted) is presumptively ruled out by the evidence reviewed in the first part of the paper (an argument originally advanced by Jolliffe, Shannon and Smith (1932b)).

The aglomerular kidney of both *Lophius piscatorius* and *Opsanus tau* (Richards, Westfall and Bott (1934); Shannon (1934b)) is incapable of secreting inulin. Since we hesitate to argue from the fish to the mammalian kidney, there remains the necessity of adducing independent evidence that the disparity between the inulin and xylose clearance is not due to secretion of the inulin. Evidence on this point is, we think, available in the facts that the amount of inulin excreted per unit of time is linearly related to the plasma level, and that the curves relating these terms extrapolate to the intersection of the coordinates. We previously deemed that this linear relationship did not necessarily exclude secretion (Shannon, Jolliffe and Smith (1932)), because we believed it possible that a secretory mechanism might be able to handle a substance in direct proportion to the plasma concentration. On reconsideration, however, we note that those substances for which there is independent evidence of secretion are found, in fact, to show a curvilinear relationship, i.e., phenol red in dog (Marshall and Vickers (1923); Marshall (1931)), frog (Marshall and

Crane (1924)), toadfish (Marshall and Grafflin (1932); Bieter (1933)); creatinine in the toadfish (Marshall and Grafflin (1932)), dogfish (Shannon (1934a, 1934b)), and in man according to evidence to be presented later. In theory, it would seem that any process of secretion, involving as it does the expenditure of energy in work, might be diminished, relative to the plasma level, at high plasma concentrations, and consequently that the amount excreted per unit time would be related to the plasma level in a curvilinear manner (Shannon (1934a)). A possible exception to this argument might occur in the instance of (a) a substance that is partly secreted, but of such a nature that a constant fraction of blood going to the kidney is actually completely cleared of it, and (b) a substance of such a nature that at high plasma levels the secreted portion is quantitatively negligible in comparison with the quantity excreted by filtration. In both these cases the amount excreted will be linearly related to the plasma level, and extrapolation from high plasma levels will lead to the zero axis. It is, of course, impossible to exclude inulin from one of the above classes, because we have no knowledge of what constitutes a high or low plasma level in relation to the cells that might be capable of secreting it. But the inulin clearance has been studied over ranges of plasma level from 50 to 400 mgm. per cent, and in view of the linear relationship existing here it would appear that if there is any secretion, the secreted moiety is small.

Although no single line of evidence is so complete as to establish proof, the fact of the linear relation between plasma level and rate of excretion, coupled with the inability of the aglomerular kidney to secrete inulin and the evidence against secretion of carbohydrates in general by the mammals, as reviewed at the opening of this paper, encourages us to proceed on the assumption that inulin is not secreted in man, and that of the above possibilities (a) can be eliminated. There is, then, no other alternative but to suppose (b) that the inulin clearance constitutes a measure of glomerular filtration, or (c) that the level of glomerular filtration lies somewhere above the level of the inulin clearance.

In either case it follows that a significant portion of the xylose is reabsorbed from the glomerular filtrate during its passage down the tu-

the exact quantity being undeterminable until there is a selection between alternatives stated above (*b* or *c*). (These investigations have not included sucrose, but since the xylose and sucrose clearances are so nearly equal in man there is probably some reabsorption of the latter as well.) The alteration of the xylose clearance toward that of inulin (0.79 to 0.89) under the influence of phlorizin seems to indicate that, in part at least, this reabsorption is an active one in the same sense as is that of glucose in the normal kidney. The absence of complete identity between the xylose and inulin clearances after phlorizin may, in this view, indicate the passive diffusion of a small amount of xylose from the tubular urine into the blood or lymph. This possibility is supported by the fact that the glucose clearance agrees with the xylose rather than with the inulin clearance. If this interpretation is accepted, it can be inferred that the passive diffusion of inulin (molecular weight = 972 or more) is small, since the clearances of the much more diffusible xylose and glucose (molecular weight = 150 and 180) after phlorizin are only 10 per cent lower than that of inulin. If the molecular weight of inulin is greater than 972, this argument is all the more effective.

It was suggested in (*c*) that there might be some active reabsorption of inulin. Even if this reabsorption were blocked by phlorizin it would be obscured by the general fall in clearances and therefore would not be revealed without an independent standard of reference. And having uncovered in these observations, and in observations on the dogfish, an active reabsorption of xylose, it becomes necessary to rule out the possibility of the active reabsorption of inulin by further evidence before it can be accepted that the inulin clearance is a measure of glomerular filtration. The constancy of the urea/inulin clearance ratio (or the lowering of the urea/xylose clearance ratio) before and after phlorizin in our data would indicate that this drug affects the xylose rather than the inulin clearance, but since the relative change in these clearances is so small, and since there is a significant fall in all the clearances after phlorizin, this argument is dubious. Observations made since the work of Jolliffe, Shannon and Smith (1932*a*) have shown that the urea/xylose clearance ratio may be altered unpre-

dictably by changes in absolute clearance, and perhaps by other factors.

Further evidence against the possible active reabsorption of inulin will be presented in a subsequent paper.

#### SUMMARY

1. The excretion of the polysaccharide inulin (the molecular weight of which is variously given as 972 to 4860) has been examined in man after intravenous infusion, and the inulin clearance has been compared with simultaneous urea, glucose and xylose clearances in both normal and phlorizinized man.

2. The inulin clearance in normal man is independent of the plasma concentration, and the curves relating plasma concentration to quantity excreted per unit time extrapolate to zero coordinates.

3. This fact, combined with the fact that inulin is not secreted by the aglomerular fish and with other evidence indicating that the mammalian kidney cannot secrete carbohydrates in general, supports the tentative conclusion that inulin is not secreted by the renal tubules in man.

4. The inulin clearance exceeds the simultaneous xylose clearance in normal man by an average of 22 per cent.

5. In the light of (3) it follows that some xylose (and sucrose) is normally reabsorbed by the renal tubules from the glomerular filtrate; this reabsorption is in part an active process, since the disparity between the inulin and xylose clearances is in part removed by phlorizin; that fraction of the disparity (10 per cent) which is not removed by phlorizin may be due to passive diffusion of the xylose from the tubular urine back into the blood or lymph.

6. It seems scarcely probable that in the normal kidney there is any significant diffusion of inulin, even accepting a molecular weight as low as 972, but in view of the fact that evidence has been uncovered for the active reabsorption of xylose, evidence against the active reabsorption of inulin should be obtained before the inulin clearance is accepted as a measure of glomerular filtration.

We wish to express our indebtedness to the Department of Medicine for the services of the

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# THE RENAL EXCRETION OF CREATININE IN MAN

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In a previous paper the evidence on the excretion of carbohydrates by the toadfish, goosefish, dogfish, dog and man was reviewed and it was concluded that, until evidence to the contrary is adduced, all the available facts are best interpreted on the supposition that pure carbohydrates in general are not secreted by the renal tubules of any of the above-mentioned forms (Shannon and Smith, 1935).

The evidence on creatinine points in the contrary direction. Although this substance is not normally present in significant quantities in the urine of aglomerular fishes, administered creatinine is copiously excreted by the toadfish and the goosefish (Marshall, 1930, 1934). Marshall and Grafflin (1932) have shown in the toadfish that the quantity of creatinine excreted per unit time does not increase in direct proportion to the quantity injected into the body. This failure to obtain direct proportionality between the quantity of substance administered and the rate of its excretion was first described for any substance by Marshall and Crane (1924) in their examination of the excretion of phenol red by the dog, and these investigators pointed out that the observed curvilinear relationship between plasma level and rate of excretion was incompatible with excretion by exclusive filtration. A similar phenomenon has subsequently been shown by Bieter (1933) and Marshall and Grafflin (1932) to occur in the excretion of phenol red in the toadfish.

An analysis of the simultaneous excretion of creatinine and xylose (or sucrose) in the dogfish by Shannon (1934a) has disclosed that the creatinine clearance at low plasma levels of this substance is from 4.2 to 7.2 times as large as is the simultaneous xylose clearance. As the plasma level is raised the creatinine clearance is depressed, both absolutely and relative to the clear-

ance of the sugar, until at plasma levels of 140 mgm. per cent the creatinine/sugar clearance ratio is less than 2. Entirely similar results have been obtained more recently in a comparison of the creatinine clearance with the inulin clearance (Shannon, 1934b). Phlorizin lowers the creatinine clearance both absolutely and relative to the sugar clearance, as first described by Clarke and Smith (1932), who ascribed this result to a depressing action of this drug on the secretory power of the tubules.

MacKay (1929-30) has shown in one individual that, under controlled conditions in man, the rate of excretion of creatinine is directly proportional to plasma concentrations up to 15 mgm. per cent; and Cope (1931) has obtained a similar result in another individual up to 8 mgm. per cent. We have not reexamined the matter within this range, but the above data, combined with those of Jolliffe and Chasis (1933) who worked between 2.4 to 11.6 mgm. per cent in one instance, and 1.8 to 7.0 mgm. per cent in a second, would seem to indicate that in man proportionality between plasma level and rate of excretion holds up to 10 mgm. per cent. This point will be discussed later.

The present paper reports observations on the excretion of creatinine in relation to higher plasma concentrations in man, and the comparison of creatinine clearances with simultaneous inulin clearances (see Shannon and Smith (1935)). The experimental procedure is identical with that described by Shannon and Smith, and in fact many of the creatinine observations were made at the same time when the other clearances described in that paper were measured. The subjects were volunteers from the wards of the Third Medical Division of Bellevue Hospital, without history or present evidence of renal pathology, having been admitted for entirely minor complaints.

We find in man, as in the dogfish, that as the plasma concentration is progressively raised above

<sup>1</sup> This paper is based on a thesis to be presented to the Graduate School of New York University in partial fulfillment of the requirements for the degree of Doctor of Philosophy.



10 mgm. per cent there is a reduction in the creatinine clearance. Since this reduction might be due to a reduction in the rate of filtration, it is necessary to have information on the simultaneous clearance of some other substance. For this reason we have included inulin clearances which, according to the available evidence, are close to the glomerular level (Shannon and Smith (1935)). Because of the spontaneous variations in renal activity that are known to occur in man as well as in other mammals, and because of the variations in renal activity in different individuals, it is convenient to express the results in terms of the creatinine/inulin clearance ratio. It should be noted, however, that in the present series of observations there has been little change in the inulin clearance in any one experiment.

Data illustrating the effects of raising the plasma concentration of creatinine upon the creatinine clearance are given in Table I. In Figures 1 and 2 are given additional data on the creatinine/inulin clearance ratios in two individuals in whose plasma the concentration of creatinine was raised from 5 to 10 mgm. per cent to intermediate, and then to high level, by two intravenous infusions of this substance. Each open circle represents the average of the creatinine/in-

ulin clearance ratio in 3 successive clearance determinations, each clearance determination being based on a urine collection period of 10 to 20 minutes. The open circles connected by a line represent successive observations made on one day. The change in creatinine/inulin clearance ratio is due to variation in the clearance of creatinine rather than that of inulin. The inulin clearance is, in fact, quite independent of the immediate plasma level of the substance, as was shown by Shannon and Smith (1935), as well as being independent of the initial plasma level. For example, in one individual (W. F.) the inulin clearance varied only from 73 to 80 when the initial plasma level of inulin obtained on six separate occasions varied from 175 to 415 mgm. per cent, and in another individual (J. S.) the clearance remained constant at 140 in two groups of observations in which the initial inulin plasma level was 115 and 400 mgm. per cent. These points are further substantiated by the summarizing data given in Table II.

At low plasma concentrations of creatinine (7.3 to 13 mgm. per cent) the creatinine clearance is from 30 to 45 per cent higher than the inulin clearance, the mean value being 1.39. This corresponds to a creatinine/xylose clearance ratio of

TABLE I

*Typical protocol of observations on J. C. showing depression of creatinine clearance resulting from raising plasma creatinine concentration. May 25, 1934. S.A. = 1.63 sq. m.*

Period number	Duration	Urine flow	Plasma			Clearance			Clearance ratios		
			Xylose	Inulin	Creatinine	Xylose	Inulin	Creatinine	Xylose Inulin	Creatinine Inulin	Creatinine Xylose
	minutes	cc. per minute	mgm. per cent	mgm. per cent	mgm. per cent	cc. per minute	cc. per minute	cc. per minute			
After 15 grams creatinine by mouth											
1	13	4.23	75.8	289	9.7	85.0	107.5	146.5	0.79	1.36	1.72
2	11	4.45	80.0	249	10.1	83.0	113.0	153.0	0.73	1.35	1.85
3	13	4.77	84.0	220	10.6	89.5	115.0	156.5	0.76	1.36	1.75
After 10 grams creatinine intravenously											
4	12	6.41	86.5	149	50.0	85.0	112.0	144.0	0.76	1.28	1.70
5	12	4.82	87.6	128	48.8	83.0	108.5	138.5	0.76	1.28	1.68
6	11	4.55	87.6	114	37.9	85.0	116.0	134.0	0.73	1.26	1.72
After 30 grams creatinine intravenously											
7	12	8.66	73.6	268	111.0	80.5	105.0	119.0	0.77	1.13	1.47
8	13	7.82	67.2	226	91.3	92.4	119.0	136.0	0.78	1.13	1.47
9	11	6.1	63.3	189	81.3	85.3	111.0	127.0	0.77	1.15	1.49

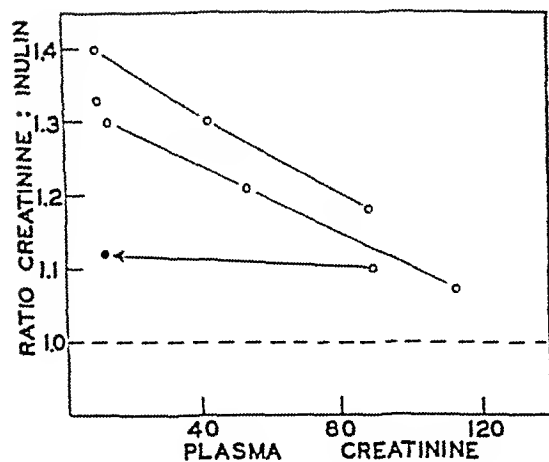


FIG. 1

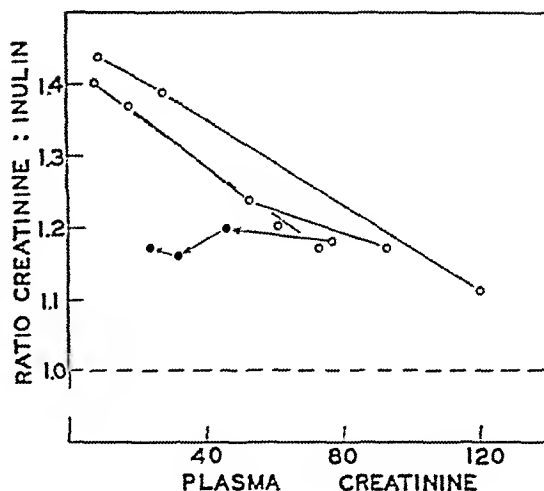


FIG. 2

FIGS. 1 AND 2. THE EXCRETION OF CREATININE IN RELATION TO PLASMA LEVEL

The ratio of the creatinine clearance divided by the simultaneous inulin clearance is plotted against the plasma concentration of creatinine in mgm. per cent. The open circles each represent the average of 3 clearance periods, observed in the order of increasing plasma concentration. The solid dots represent the average of 3 clearance periods when they are observed after the plasma concentration has been raised and allowed to fall again. Points connected by lines represent observations on a single individual in one day.

TABLE II

Summary of absolute clearances on W. F. and S. C. showing that the variation in the creatinine/inulin ratio is due to changes in the absolute creatinine clearance, the inulin remaining essentially constant: these clearances are those represented as open circles in Figures 1 and 2.

	W. F.			S. C.		
	Number of observations	Plasma mgm. per cent	Average clearance cc. per minute	Number of observations	Plasma mgm. per cent	Average clearance cc. per minute
Inulin	2	>400	80	6	>300	114
	7	301-400	76	8	201-300	109
	10	201-300	75	7	101-200	97
	11	<201	77	3	<101	109
Creatinine	5	>91	80	3	>99	121
	6	71-90	90	6	71-99	116
	7	41-70	91	5	41-70	127
	6	21-40	105	7	12-40	143
	6	1-10	112	3	10-11	164

1.62 to 1.88, as compared to the average figure of Jolliffe and Chasis (1933) of 1.73. But as the plasma concentration of creatinine is raised the creatinine clearance falls absolutely and relative to the inulin clearance, until at plasma concentrations of 96 to 127 mgm. per cent the creatinine clearance exceeds the inulin clearance by a mean of 12 per cent.

It should be noted particularly that the first observations in the series described above were

made at low plasma concentrations; an infusion of a moderate amount of creatinine was then given and a second set of 3 clearances was obtained; then, after the infusion of creatinine a second time, a third series of clearances was obtained. This procedure was so standardized that the periods of observation were completed within one hour after the injection of the creatinine.

When the creatinine/inulin clearance ratio is determined on a "falling" blood curve, that is,

over an extended period of time after the injection of a considerable quantity of creatinine, a different result is obtained. The creatinine/inulin clearance ratio does not retrace the course set on a "rising" plasma curve, but remains at a depressed level for a number of hours, as is indicated by the solid dots in Figures 1 and 2. (No such phenomenon is observable in the case of inulin as has been pointed out above.) The normal level of creatinine clearance is restored within a few days, but just how fast we have not determined. A search for this phenomenon in the dogfish failed to reveal it; the creatinine clearance rises in this animal, relative to the sugar clearance, as fast as the plasma creatinine falls, so that identical clearances are obtained at low plasma concentrations, before and after the administration of large doses of this substance. But in the dogfish several days are required for the plasma concentration to return to its former level, and it is possible that this time is necessary for the secretory mechanism to return to its initial state (Shannon (1934a)). The practical significance of this delayed recovery needs to be emphasized, since this apparent constancy of the creatinine/inulin clearance ratio when the plasma level of creatinine is falling is also evident in successive periods of urine collection such as have been used in obtaining each point in Figures 1 and 2. It seems probable, therefore, that our creatinine/inulin clearance ratios, as recorded in these figures, are really too low, being "set" or determined by a plasma level that was initially higher than that actually observed.

The data given in Table I will enable the reader to judge the magnitude and significance of all the terms involved. Xylose has not been present in every instance, but when present there has been no change in the inulin/xylose clearance ratio occasioned by the administration of the creatinine. This fact emphasizes that it is its own clearance that is affected by the administration of creatinine, rather than that of inulin, the clearance of reference.

The question may be raised, to what extent does the excretion of endogenous creatinine enter into the results described above. Table III contains data on the excretion of apparent endogenous creatinine (total chromogenic substance) in

TABLE III

*The excretion of endogenous creatinine in man*

Subject	Number of periods	Average urine flow	Average apparent creatinine	
			Plasma level	Clearance
		<i>cc. per minute</i>		
R.C.....	2	8.4	1.44	109.0
H.S.....	2	16.6	1.32	120.0
C.C.....	2	9.5	1.44	134.5
B.K.....	2	4.0	1.70	88.5

four normal individuals. The clearance of endogenous creatinine is such that, if a correction were made for it in the data displayed in Figures 1 to 3, it would raise slightly, but not significantly, both the total creatinine clearance and the creatinine/inulin clearance ratio at low plasma levels, while the correction would have no effect at higher plasma levels.

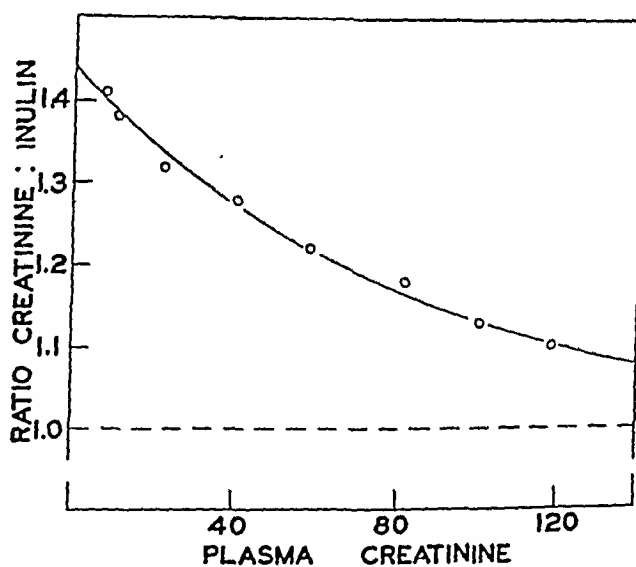


FIG. 3. WHEN 80 OBSERVATIONS ON THE CREATININE/INULIN CLEARANCE RATIO ARE AVERAGED IN GROUPS OF TEN, SELECTED IN ORDER OF INCREASING PLASMA CREATININE CONCENTRATION, AND PLOTTED AGAINST THE LATTER, THE 8 POINTS SO OBTAINED FALL UPON A SMOOTH CURVE WHICH APPEARS TO BE APPROACHING 1.0 AS THE ASYMPTOTE.

The curve given in this figure is calculated by the exponential equation described in the legend of Figure 4, and represents the relationship between  $1 + y$  (creatinine/inulin clearance ratio) and  $x$  (plasma creatinine level). This curve indicates that the creatinine clearance is driven down towards the level of the inulin clearance by raising the concentration of creatinine in the plasma at such a slow rate that the asymptote could not be reached in practice.

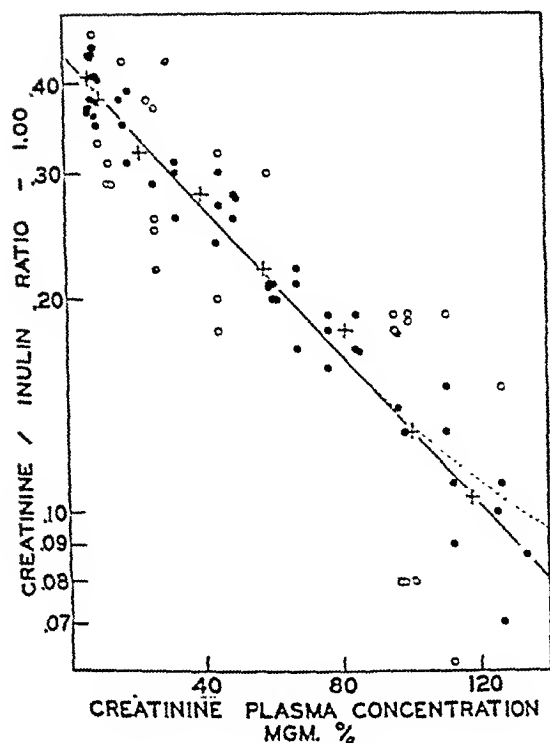


FIG. 4. THE RELATIONSHIP OF CREATININE/INULIN CLEARANCE RATIO TO PLASMA LEVEL OF CREATININE AS OBTAINED FROM 80 OBSERVATIONS MADE UNDER STANDARDIZED CONDITIONS AS DESCRIBED IN THE TEXT.

When the term,  $\text{creatinine/inulin} - 1.0 = (y)$ , is plotted against the plasma level of creatinine ( $x$ ), an exponential relationship of the type  $y = ae^{-\alpha x}$  is obtained, where  $a$  is the intercept on the  $y$  axis (.44) and  $\alpha$  is a constant determining the slope of the line (.0122). The solid line indicates the relationship described by the equation, the term  $y$  in this figure being plotted on a logarithmic scale.

With regard to this exponential relationship, we note the following: if the term  $1 + y$ , as generated by the above equation, be taken as the mean, and if the standard deviation from this mean be calculated in terms of per cent of the mean, it is found to be 3.6 per cent. All observations fall within 3 times the standard deviation. The agreement is equally good for both the upper and lower portions of the curve. In the figure those points falling within once the standard deviation are indicated by solid dots, those falling within twice the standard deviation by open circles, and those within three times by half-filled circles. The crosses indicate the average data plotted as open circles in Figure 3. When the limitations of experimental and chemical methods are considered, this agreement is as satisfactory as can be expected. So far as the physiological significance of this equation is concerned, perhaps the most noteworthy point is the fact that the quantity of creatinine apparently secreted per minute per 100 cc. glomerular filtrate (inulin clearance) passes through a maximum of 13.3 mgm. at a plasma level of

70 mgm. per cent, to decrease at higher plasma levels. If, however, it is supposed that the creatinine apparently secreted per 100 cc. glomerular filtrate increases to a maximum and maintains this maximum at plasma levels above 70 per cent, then the relationship would be such as is indicated by the dotted line in the figure. Since the latter possibility does not seem unlikely, it throws doubt upon the physiological significance of the exponential relationship.

In our data there are 80 instances in which the creatinine clearance and creatinine/inulin clearance ratio were determined at two or more widely separated plasma levels of creatinine, and in which the technique was so standardized that the first period of observation was started within 15 to 30 minutes after the completion of the creatinine infusion. From these 80 instances we have constructed an average curve relating creatinine/inulin clearance ratio to plasma creatinine concentration by averaging each group of ten observations at successively higher plasma levels of creatinine. The average curve (Figure 3) thus obtained is smooth and convex to the intersection of the coordinates. It is apparent that the curve does not approach a zero clearance as its asymptote; in our interpretation, this asymptote should be the level of glomerular filtration, which we can take as approximately the inulin clearance. This curve is apparently exponential in character and is discussed in the legend to Figure 4. (In plotting the mass data in this figure, the plasma level obtaining in the first of each group of three observations was taken for all three, since the creatinine clearance is "set" by the highest plasma level obtained.) In view of the general relationship it appears that lowering the plasma level of creatinine from 10 to 0 mgm. per cent would have an insignificant effect upon the absolute creatinine clearance (increasing the creatinine/inulin clearance ratio from 1.39 to 1.44). This fact may explain why the several observers mentioned above failed to find any relationship between creatinine clearance and plasma creatinine within this range.

#### *Action of phlorizin*

The knowledge that phlorizin depresses the creatinine clearance in the dogfish to levels close to the sugar clearances (Clarke and Smith (1932); Shannon (1934a, 1934b)) has led us to believe that, if the creatinine clearance in excess

of the inulin clearance in man is really a secretory phenomenon, then phlorizin should produce a similar result in this case. Chasis, Jolliffe and Smith (1933) failed to obtain any significant effect upon the creatinine/xylose clearance ratio in man by the administration in several individuals of amounts of phlorizin up to 20 mgm. per kilogram, and in a single instance of 65 mgm. per kilogram. A negative result was also obtained by Goldring and Welsh (1934) following the administration of much larger doses of this drug by stomach. Believing that the quantity hitherto administered was short of the required amount, we raised the intravenous dose to 100 mgm. per kilogram (see Shannon and Smith (1935)). A summary of all data before and after phlorizin is given in Table IV and Figure 5. All clearances usually fall in consequence of the intravenous administration of phlorizin; this transient depression is apparently circulatory or glomerular in origin, and has been noted by Marshall and Grafflin (1932) in the sculpin, by White and Monaghan (1933) in the dog, and by Chasis, Jolliffe and Smith (1933) and Goldring and Welsh (1934) in man after intravenous and oral administration. Pitts (1934) has found that the creatinine (and creatine) clearance in the dog falls after phlorizin, but not the xylose clearance; the creatinine/creatinine clearance ratio, however, remains constant. In view of this frequent de-

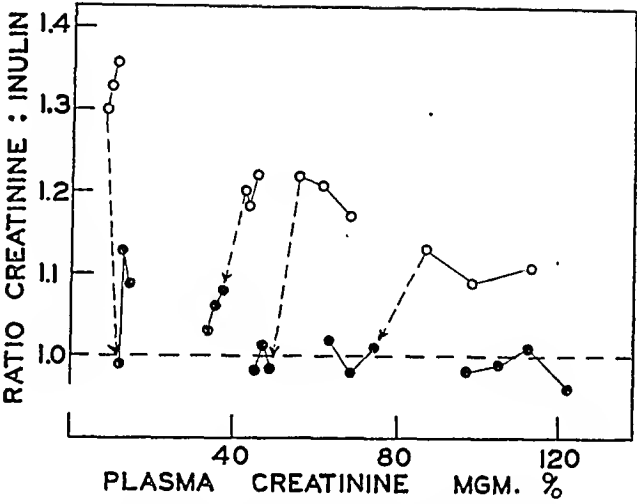


FIG. 5. THE INFLUENCE OF PHLORIZIN (SOLID DOTS) IN REDUCING THE CREATININE CLEARANCE TO THE INULIN CLEARANCE.

Phlorizin was given intravenously (100 mgm. per kilogram) after the 3 control clearances (open circles) were observed.

pression of all clearances, it is impossible to decide from the absolute values of a particular clearance whether the excretion of that substance is specifically affected by the drug. It is none the less significant that, under the action of adequate doses of phlorizin, the creatinine and inulin clearances are brought together perfectly at the high plasma levels of creatinine, if our observations on single individuals are significant, and less perfectly at low plasma levels. Thus, in the data

TABLE IV

Summary of observations of men before and after phlorizin (100 mgm. per kilogram intravenously). Each datum given is the average of 3 clearance periods of 10 to 15 minutes each

Subject	Average urine flow	Average plasma creatinine concentration	Average clearances					Average clearances/inulin clearance			
			Urea	Xylose	Inulin	Creatinine	Glucose	Urea	Xylose	Creatinine	Glucose
	cc. per minute	mgm. per cent	cc. per minute	cc. per minute	cc. per minute	cc. per minute	cc. per minute				
S.C.	4.0	9.6	63.2	91.7	123.0	163.8		0.514	0.745	1.33	
	3.1*	12.2	35.6	57.1	66.9	71.6	56.2	0.532	0.854	1.07	0.840
B.R.	6.0	43.7	55.9	69.4	84.1	101.0		0.675	0.826	1.20	
	4.5*	35.5	47.1	61.7	69.6	73.3	61.5	0.677	0.887	1.05	0.884
W.F.	10.9	61.5	49.6	61.0	75.8	91.1		0.654	0.804	1.20	
	4.5*	47.2	35.6	51.5	56.3	55.7	50.4	0.632	0.916	0.99	0.896
J.C.	4.4	99.0			112.0	125.0				1.12	
	3.7*	68.3			100.0	101.0				1.01	
B.R.	7.4*	109.7		59.5	67.8	66.6	61.0		0.878	0.98	0.900

\* Indicates after phlorizin.

given in Figure 4 the average creatinine/inulin clearance ratios of 3 clearances after phlorizin are 1.07 at 12 mgm. per cent, 1.06 at 36 mgm. per cent, 1.00 at 47 mgm. per cent, 1.00 at 68 mgm. per cent and 0.98 at 109 mgm. per cent.

#### DISCUSSION

It is clear from the above observations that the creatinine clearance is depressed, both absolutely and relative to the inulin clearance, by an elevation of the plasma concentration of creatinine. This result leads, of course, to a reduction in the rate of excretion relative to plasma level, the phenomenon described in the first part of the paper. As Marshall and Crane (1924) pointed out in their discussion of the excretion of phenol red by the dog, a curvilinear relationship between plasma level and rate of excretion (or, in our present terms, between plasma level and clearance) is incompatible with exclusive filtration. It is conceivable that a substance excreted both by filtration and tubular secretion might display a linear relationship in this respect (as in fact creatinine does when the plasma is falling); but we are unable to imagine any reason why the clearance should decrease with increasing plasma concentration unless secretion is involved. If filtration alone were operative, this result would require that either the quantity of creatinine filtered decreased, or that the relative amount of creatinine reabsorbed (if any) increased, as the plasma level rose. A positive finding of a depression of clearance caused by increased plasma concentration can therefore be taken in our opinion as positive evidence of secretion. We offer this, consequently, as independent evidence of the secretion of creatinine by man.

The nature of the creatinine, excreted in excess of the amount that can be accounted for by glomerular filtration (inulin clearance), is unknown. It might be true creatinine added to the post-glomerular filtrate by tubular activity; or it might be a creatinine derivative that gives the Jaffe reaction, formed prior to or during tubular secretion. No method is available at this time to distinguish these possibilities.

Shannon and Smith (1935) have presented evidence that the rate of glomerular filtration lies at least as high as the level of the inulin clearance,

but they have pointed out that there is nothing to indicate the absence of active reabsorption of this substance; and in view of the fact that evidence is at hand for the active reabsorption of xylose (and sucrose), such evidence is needed before the active reabsorption of inulin can be ruled out. There is no reason to believe that creatinine would influence either the active reabsorption or the diffusion of inulin, and, in fact, the constancy of the inulin clearance and of the inulin/xylose clearance ratio is substantial evidence that such is not the case. We present the fact that the creatinine clearance can be depressed to within 10 per cent of the simultaneous inulin clearance by the administration of creatinine, as evidence that the rate of glomerular filtration cannot lie higher than 10 per cent above the inulin clearance. It is reasonable to suppose that at the highest plasma levels observed some creatinine is still being secreted and therefore that the rate of glomerular filtration lies nearer the level of the inulin clearance than the lowest level of the creatinine clearance.

The statement in the above paragraph would have to be qualified if creatinine were reabsorbed to any extent. But the fact that phlorizin lowers the creatinine clearance to, but not below, the inulin clearance, indicates that under the conditions of these experiments there is no measurable reabsorption of creatinine, and that the creatinine clearance in phlorizinized man (though only under this condition) is at the level of glomerular filtration.

#### SUMMARY

1. When the creatinine concentration of the plasma is raised the creatinine clearance in man is depressed, both absolutely and relative to the simultaneous inulin clearance. At plasma levels of 7.3 to 13.0 mgm. per cent the mean ratio of the creatinine clearance over the inulin clearance is 1.39. At plasma levels from 96 to 127 mgm. per cent this mean ratio is lowered to 1.12.

2. The relationship expressed in (1) is believed to be independent evidence of the secretion of creatinine by the renal tubules in man.

3. It is suggested that the level of glomerular filtration in normal man lies between the lowest creatinine clearance and the inulin clearance obtained at high plasma levels of this substance; and

in view of the fact that at the highest plasma levels observed the creatinine clearance is still unquestionably elevated by secretion, it is probable that the level of glomerular filtration lies closer to the inulin clearance than to the former. This is believed to be independent evidence against any extensive active reabsorption of inulin.

4. Phlorizin administered in doses of 100 mgm. per kilogram brings the clearances of creatinine and inulin together, presumably by a specific depressant action on the tubular secretion of the former substance. The identity of these clearances under phlorizin is evidence against the diffusion of creatinine under the conditions of the experiment.

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# THE ELECTROLYTE BALANCE IN ACUTE GOUT<sup>1</sup>

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The classification of gout as a disturbance of metabolism is probably as old as the recognition of such disorders in medicine. The ancient physicians who wrote about *podagra* suggested a disorder of normal metabolism when they attributed the cause of the disease to a disturbance of the humours of the body. But it was not until 1797 that Wollaston (1) offered experimental evidence for the validity of this assumption when he identified sodium urate crystals in the material obtained from the tophi of patients with gout. Some time later Garrod (2) extended this observation and noted an increased concentration of uric acid in the blood from patients similarly afflicted. The classification of uric acid as a purine body by Emil Fischer (3) completed one phase of the pathologic physiology that confirmed the earlier impressions that gout should be placed in the category of metabolic dyscrasias.

It is usually granted by contemporary observers that an integral part of the pathogenic process in gout is a disturbance of uric acid metabolism. Most arguments that have arisen about this subject have been over the question as to whether the disturbance is one of uric acid formation, excretion, or destruction, rather than a denial of the major premise. Exceptions to this statement have been made by the German investigators Grafe (4) and Thannhauser (5), who have advanced the possibility that the disturbance of uric acid metabolism in gout may be secondary to a pathologic process more general in effect.

The data presented in this paper offer support to the hypothesis that gout may be a disturbance of the equilibrium of the body more widespread in scope than a dysfunction of the uric acid metabolism. We accept the classification of gout as a metabolic disease, but our reasons for this acceptance are based upon data only casually related to

a disturbance of purine metabolism. These data have been obtained from an extended study of the mineral and water exchange associated with attacks of acute gout in the patient F. M. We have also included confirmatory data obtained from a study of a second patient, K. H.

## METHODS

The protocols of the two cases studied are presented at the end of this communication together with the records of the food ingested. During the periods of study in the metabolism ward of the Massachusetts General Hospital, the patients were given a low-purine diet of constant amount. The food was purchased in 5-day lots according to the method of Atchley, Loeb et al. (6). One-half of a duplicate day's diet from each lot was weighed, dried and analyzed. On the days of severe symptoms all of the food was not eaten, and the fluid intake was increased. For these days, food equal to the amounts consumed was taken from the 5-day lots; it was dried, analyzed, and the data with respect to intake corrected accordingly. The urine was collected under toluol and kept in the ice chest. It was partitioned and analyzed each 24 hours. The stools, which were collected in periods corresponding to the symptoms, were dried and analyzed similarly to the diets. Rectal temperatures were recorded 4 times a day.

The methods employed for the analyses of the blood, and the correction of the concentration of the constituents of the serum to  $pH_7 = 7.45$ , have been described in most instances elsewhere (7). The uric acid was determined according to the method of Folin (8) on plasma or unlaked whole blood. The inorganic base was determined on a portion of urine, stool, or diet, treated with a few drops of  $H_2SO_4$ , ashed in a platinum evaporating dish and taken up in 0.5 N HCl. Serum was ashed with  $HNO_3$ ,  $H_2SO_4$ , and superoxol in a silica tube, and the sodium was determined ac-

<sup>1</sup> Presented in abstract form at the meeting of the American Society for Clinical Investigation, April 30, 1934.



according to the method of Butler and Tuthill (9). Potassium was determined by the method of Jacobs and Hoffman (10). The pH of the urine was determined colorimetrically by the bi-color method of Hastings et al. (11), and the titratable acid according to the method of Folin (12).

## OBSERVATIONS

The experimental data presented at this time were obtained from two patients suffering from recurrent attacks of acute gout. The patient F. M. was under our clinical supervision for more than 22 months. During this time he had 50 attacks of acute gout, 21 of which were observed while he was on a metabolic regime. The significant changes observed in the attack described in this paper correspond qualitatively to the changes observed during the other 20 attacks. The patient K. H. was observed over a period of 9 weeks, and during this time she had 7 attacks of acute gout.

The duration of study of these patients was very long, and all of the data accumulated could not be adequately treated by the methods in common use. It was necessary and convenient, therefore, to divide the long periods of study. The frequent occurrence of attacks of acute symptoms allowed us to employ these attack periods as points for division. The selection of one phase of an attack, as judged from the clinical condition of the patient, is not difficult and may be repeated for each attack. The time elapsed from one phase of an attack to the same phase of a following attack has been called by us a *cycle*. This cycle may be subdivided into 3 periods, following the scheme suggested by Duckworth (13). These are the *prodromal*, *attack* and *recovery* periods, respectively. The *prodromal* period follows the last days of the recovery from an attack and includes the first hours or day of mild symptoms of the subsequent attack. The usual duration of the prodromal period is from 2 to 4 days. With the progression of symptoms the *attack* period begins and the patient is confined to bed. When the symptoms reach their maximum, specific medication is required. Colchicin was the specific drug given during the attacks herein described. This drug was given in divided doses over several hours and in a sufficient amount to induce diar-

TABLE I  
Intake and urinary data on the patient F. M.

Date *	Gout severity	Rectal temperature ° C.	Weight kgm.	Urine														Intake								Period																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																												
				Volume cc.	pH	Sodium m. eq.	Potassium m. eq.	Calcium m. eq.	Total inorganic base m. eq.	Ammonia m. eq.	Titratable acid m. eq.	Chloride m. eq.	Phosphate m. eq.	Urate m. eq.	Urate mgm.	Total nitrogen grams	Fluid cc.	Sodium m. eq.	Potassium m. eq.	Calcium m. eq.	Total inorganic base m. eq.	Chloride m. eq.	Phosphate m. eq.	Total nitrogen grams																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																														
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\* The following drugs were given: 8-9, codeine sulphate 100 mgm.; 9-10, codeine sulphate 100 mgm.; pantopon 20 mgm., phenobarbital 100 mgm.; 10-11, pantopon 20 mgm., phenobarbital 100 mgm.; 11-12, codeine sulphate 100 mgm., colchicin 10 mgm., barbitol 300 mgm.

rhœa. With the onset of diarrhea, the symptoms subside and the attack period is considered over. The recovery period extends from the subsidence of symptoms to the prodromal period of the next cycle. The apparent merging of the recovery period of one cycle with the prodromal period of the following cycle prevented us from obtaining a normal or control period. Subsequent observations of other patients with gout have strengthened our earlier assumption, that it is doubtful whether a normal period of significant duration is ever observed in patients with gout. The failure to obtain a control period necessitated certain assumptions, which will be discussed below.

In addition to the specific medicine that was administered during each cycle, sedatives and hypnotics were given. These are enumerated in the tables for the respective patients. The action of all of the drugs used in this study on the acid-

base excretion was investigated in F. M. during a period of freedom from symptoms. The changes in the water and salt metabolism following the administration of these drugs may be neglected for the present discussion and will be presented elsewhere.

#### *The electrolyte balance for the patient F. M.*

The paroxysms of acute gout in this patient were very frequent, and the data obtained from the several studies that extended over many weeks describe a continual change of the internal environment of the body. In Tables I, II, and III are given the pertinent data of the electrolyte balance for a 12-day cycle, chosen because it seemed to be typical. Changes of varying magnitude were observed in other cycles, but this cycle has the advantage of showing most of the variations

TABLE II

*Electrolytes excreted in the stools and withdrawn in the blood on the patient F.M.*

Date	Stool						Blood		
	Dry weight	Sodium	Potassium	Calcium	Chloride	Total nitrogen	Sodium	Potassium	Chloride
<i>December, 1933</i>	<i>grams</i>	<i>m. eq.</i>	<i>m. eq.</i>	<i>m. eq.</i>	<i>m. eq.</i>	<i>grams</i>	<i>m. eq.</i>	<i>m. eq.</i>	<i>m. eq.</i>
7-8	10	9.0	7.0	17.8	3.7	0.56	3.6	1.5	3.3
8-9	10	9.0	7.0	17.8	3.7	0.56	3.6	1.5	3.3
9-10	10	9.0	7.0	17.8	3.7	0.56	3.4	1.4	3.1
10-11	10	9.0	7.0	17.8	3.7	0.56	3.4	1.4	3.1
11-12	10	9.0	7.0	17.8	3.7	0.56	3.7	1.5	3.2
12-13	14	4.1	29.0	15.9	1.1	0.67	3.6	1.5	3.1
13-14	14	4.1	29.0	15.9	1.1	0.67	3.7	1.5	3.3
14-15	26	2.0	12.0	43.2	11.7	1.22	3.6	1.5	3.1
15-16	26	2.0	12.0	43.2	11.7	1.22			
16-17	26	2.0	12.0	43.2	11.7	1.22	3.6	1.5	3.2
17-18	26	2.0	12.0	43.2	11.7	1.22			
18-19	26	2.0	12.0	43.2	11.7	1.22	3.7	1.6	3.1
19-20	26	2.0	12.0	43.2	11.7	1.22			
20-21	26	2.0	12.0	43.2	11.7	1.22	3.5	1.4	3.1

TABLE III

*Summary of certain balance data obtained on the patient F.M.*

	Prodromal period			Attack period			Recovery period			Total of the three periods		
	In-take	Out-put	Daily balance	In-take	Out-put	Daily balance	In-take	Out-put	Daily balance	In-take	Out-put	Daily balance
Sodium, <i>m.eq.</i> .....	144	269	-62	175	270	-24	427	179	+41	746	718	+2.3
Potassium, <i>m.eq.</i> .....	153	137	+ 8	216	249	- 8	449	388	+10	818	774	+3.7
Calcium, <i>m.eq.</i> .....	80	49	+15	119	78	+10	256	261	- 1	455	388	+5.5
Chloride, <i>m.eq.</i> .....	152	278	-63	187	290	-25	425	288	+23	764	856	-7.6
Total nitrogen, <i>grams</i> .....	19.1	18.3	+ 0.4	26.5	43.4	- 4.2	56.7	51.4	+ 0.9	102.3	113.1	-0.90

noted in the blood and urine during any period of study.

The *prodromal period* began December 7th and lasted 48 hours. On the first day of this period the patient had no symptoms of acute gout and his temperature did not exceed  $37.3^{\circ}\text{C}$ . A urinary output of 2,035 cc., 25 per cent above normal, was the first warning of an impending attack. The normal excretion by this patient during the last 3 days of the recovery period and by 3 individuals without gout on a similar diet at the same season of the year was about 1,600 cc. The increased urinary output preceding an acute attack was as obvious to the patient as it was to us. This diuresis was accompanied by an increased total excretion of Na and Cl, as well as an increased concentration of both ions. The excretion of phosphate, urate, and total nitrogen in the urine was increased in amount, but their concentrations remained essentially unchanged. The total excretions of the other constituents analyzed were not increased.

The second and last day of the prodromal period of this cycle the patient had mild symptoms of gout. He had a slight pain in the proximal phalangeal joint of the right big toe, but he was ambulatory throughout the day. The white blood count was 9,500, and his temperature did not exceed  $37.3^{\circ}\text{C}$ . His weight had increased 0.5 kgm. even though the urinary output was 2,950 cc. The increased urinary excretion of Na and Cl, observed on the preceding day, reached a maximum of 124 m.eq. and 135 m.eq. respectively. The intake of these substances on this day was 72 m.eq. and 76 m.eq. respectively, which gave a negative balance of more than 50 m.eq. for each ion. Increased excretions of lesser magnitude were observed in the other urinary constituents. The K excretion increased 30 m.eq. and the titratable acid and phosphate increased 10 m.eq. The excretion of uric acid increased 3 m.eq. The excretion of all other constituents except  $\text{NH}_4$  and titratable acid was greater than was observed on any other day during the cycle. The pH of the urine was unchanged.

The *attack period* began the third morning of the cycle. The proximal phalangeal joint of the left big toe was involved in addition to that of the right big toe. The patient was confined to his bed, but his appetite remained good, and all of the diet

was consumed. Though the fluid intake was increased 200 cc., the urinary volume was 760 cc. less than on the previous day, and the weight was decreased 0.5 kgm. The concentration of Na and Cl in the urine increased, but the amounts excreted were 10 m.eq. less. The amount of  $\text{NH}_4$  excreted was unchanged, while the pH increased to 5.5. Thus on the first day of severe symptoms the pronounced changes in the electrolyte balance had passed a maximum and were rapidly diminishing.

The symptoms continued unabated the fourth day. In addition to the toes, the right sacroiliac joint and right knee were involved. The appetite began to wane for the first time during this cycle, and the intake of food was diminished by one-half. The white blood cell count increased to 13,000, and the rectal temperature to  $38.8^{\circ}\text{C}$ . This was the highest temperature observed during the cycle. Clinically, the symptomatic response by this patient on this day was the most marked of any during the cycle. In spite of the severity of symptoms, the excretion of electrolytes and water was less than on any day previously discussed. The urinary volume had decreased to 1,625 cc., and the K excretion was within 4 m.eq. of the intake. The excretions of Na and Cl had decreased about 20 m.eq., but were appreciably above the intake because of the lessened ingestion of food. The other constituents were all decreased in amount. Colchicin was started in the morning of this day, but diarrhea resulting from this drug did not begin until 24 hours afterward. It is conceivable that the excretion of electrolytes was appreciably influenced by the fluid drawn into the intestines before the onset of diarrhea.

The third morning of the attack period, the fifth day of the cycle, was associated with a partial alleviation of symptoms. The only new site of involvement was the left Poupert's ligament. Colchicin medication was continued only through the morning. Late in the afternoon diarrhea began, and 3 stools were passed before the patient retired for the night. Although the diarrhea was associated with subsiding symptoms, the patient's appetite remained poor and the intake of food was even less than on the previous day. The urinary volume of 920 cc. was the smallest observed during the cycle. The excretion of Na and Cl con-

tinued to decrease, while the excretion of K remained unchanged.

The sixth day of observation, the last day of the attack period, the rectal temperature was normal. The white blood cell count was 8,650, and the body weight had decreased 1.6 kgm. Diarrhea persisted, but symptoms of the acute attack had disappeared, and the food intake was increased to the amount taken before the acute symptoms began. The urinary volume was 1,530 cc., yet some of the constituents were excreted in amounts far below those observed in the prodromal period. The total Na excretion on this day was only 4.4 m.eq., compared with 124 m.eq. four days previously, and the Cl excretion was 125 m.eq. less than the maximum of 135 m.eq. The excretion of K was decreased by one-half. The other electrolytes showed little or no variation. On this day the urinary pH was 5.1, the lowest observed during the cycle.

The *recovery period* began on the 7th day of the cycle and continued for 6 days. During this period the rectal temperature did not rise above 37.3° C., nor did the white blood count reach 9,000. The first 2 days of this period, the urinary volume was below the amount for the last 4 days. The urinary pH remained between 5.3 and 5.5. The excretion of Na was 4.6 m.eq. on the first day of the period, which was approximately the same as that excreted on the previous day. The excretion of Cl had diminished to 7.6 m.eq. The

excretion of K was 27 m.eq. Approximately the same amount of K was lost in the stool as was excreted in the urine on this day. In contrast, the amounts of Na and Cl in the diarrheal stool were negligible. The excretion of 3.7 m.eq. of uric acid was the lowest of any day described.

The last 4 days of this period, the urinary volume was approximately 1,600 cc. daily. The excretion of K was 55 m.eq., and the excretion of Ca was slightly more than 5 m.eq. The daily excretion of  $\text{NH}_4$  varied between 20 m.eq. and 25 m.eq., and the excretion of phosphate was 5 m.eq. less than the  $\text{NH}_4$ . The end of the recovery period completes this cycle. Two additional days are included in the Tables, which comprise the prodromal period of the following attack. These 2 days, that are a part of the next cycle, are included in order to emphasize the diuresis and the salt loss in the prodromal period and to clarify the duration of the cycle just described.

In addition to the electrolytes excreted in the urine, appreciable amounts were lost in the stools or removed in the samples of blood that were drawn daily (Table II). Atchley, Loeb et al. (6) have shown that stool analyses contribute little to the knowledge of a balance experiment in its entirety. This observation was confirmed by us except on the days when there was diarrhea.

The *changes in the whole blood and serum* are given in Table IV. The white blood cell count has been discussed above as an aid in the inter-

TABLE IV  
*Experimental observations on blood and plasma on the patient F.M.*

Date	Whole blood						True plasma. Electrolytes are corrected to pH <sub>s</sub> = 7.45									
	Total CO <sub>2</sub>	Pco <sub>2</sub>	Oxy- gen ca- pacity	Cell volu- me	White blood cells	Uric acid	Bicar- bonate	Chlo- ride	Phos- phate	Urate	Pro- tein	Sod- ium	Potas- sium	Calc- ium	Total inor- ganic base	Non- protein nitro- gen
Decem- ber, 1933	m. eq. per liter	mm. Hg	m. eq. per liter	per cent		mgm. per 100 cc.	m. eq. per liter	m. eq. per liter	m. eq. per liter	m. eq. per liter	m. eq. per liter	m. eq. per liter	m. eq. per liter	m. eq. per liter	m. eq. per liter	mgm. per 100 cc.
7	22.3	44.3	9.37	46.8		7.9	24.7	102.5	1.8	.79	17.8	141.2		5.0	152.3	17.6
8	21.7	41.5		46.2	9,500	7.0	24.6	102.2	1.9	.73	16.9	142.6	3.3	5.0	156.0	21.7
9	21.6	41.7		47.2	14,250	7.8	24.6	101.6	1.7	.73	17.1	141.2			154.7	
10	21.9	37.9		45.2	13,000	6.8	25.4	100.6	1.7	.67	18.9	142.0	2.9	5.0	153.9	
11	22.3	39.6		46.2	15,750	6.5	26.3	96.0	1.6	.64	15.9	140.0	3.5	5.1	151.6	
12	23.3	43.7		47.7	8,650	9.3	27.5	96.3	1.7	.80	18.2	141.2	2.3			
13	23.3	46.2	9.52	47.7	8,200	7.3	25.3	99.3	1.5	.78	17.8	143.0	4.1		153.0	
14	21.4	36.3		44.7	8,800	9.7	26.0	100.2	1.6	.76	18.7	141.2	3.9		151.6	
16	23.8	44.5		42.0	7,400	7.3	24.7	101.8	1.7	.66	16.6	141.3	4.0	5.2	155.7	
18	23.2	40.0		41.5	8,550	8.5	26.2	102.7	2.1	.77	17.2	141.4	7.1		155.6	
20	22.5	41.5		42.3	11,000	9.0	25.2	105.1	2.0	.80	18.3	141.6	7.1		156.5	23.4

pretation of the clinical course of the acute attack. The cell volume exhibited minor fluctuations during the attack but fell appreciably during the period of recovery. This significant decrease in cell volume was infrequently observed in the other cycles that were studied. The number of determinations of oxygen capacity was insufficient to aid in the interpretation of this phenomenon. The concentrations in the serum of calcium, inorganic phosphate and urate were not appreciably influenced by the attack of gout. In the complete cycle described at this time there was no increase in the concentration of K before the attack. In the attack that followed there was an increase in concentration of K from 4.0 m.eq. to 7.1 m.eq. Such an increase was confirmed in several of the subsequent attacks. The CO<sub>2</sub> content of the serum and the concentration of Cl during the prodromal period were in the normal range, but their changes in concentration during the acute attack reached a magnitude not approached by any other electrolyte except proteinate. The Cl decreased in concentration from 102.2 m.eq. on the second day of the prodromal period to 96.2 m.eq. on the last day of the attack period. The CO<sub>2</sub> content of the serum increased 2.9 m.eq. over the same period. A decrease of 3 m.eq. in the serum sodium concentration was observed in all of the cycles in which sodium was determined. The con-

centrations of Na, total base, Cl, CO<sub>2</sub>, PO<sub>4</sub> and urate at the end of the recovery period were all within 1 m.eq. of their concentrations before the onset of symptoms.

*The electrolyte balance for the patient K. H.*

In Table V are given the various data of the electrolyte balance for this patient during a cycle of gout of 14 days' duration. There are certain features in the response of K. H. that are at variance with the response of F. M. Her weight showed a steady rise of 2.2 kgm. during the period observed. The paradoxical gain in weight, observed in F. M. before the attack, was confirmed, but the magnitude of gain was less. Likewise, a diuresis before the onset of symptoms was observed, but the amount was not as large. The maximum amount of urine excreted by K. H. was 2,120 cc. on the day of severe symptoms. This volume was 200 cc. greater than that of the preceding day and 700 cc. greater than that observed on the day before the onset of symptoms. An increased excretion of Cl, from 80 m.eq. to 135 m.eq., accompanied the diuresis. This maximum excretion does not represent as great an excess for K. H. as for F. M., because the chloride intake was somewhat greater. The excretions of urate, phosphate and titratable acid on the days when the diuresis was observed were increased in amount,

TABLE V  
*Daily observations on the patient K. H.*

Date *	Gout	Rectal temperature	Weight	Intake	Urinary constituents						Period
				Chloride	Volume	Chloride	Urate	Phosphate	Ammonia	Titratable acid	
<i>April, 1934</i>	<i>severity</i>	<i>° C.</i>	<i>kgm.</i>	<i>m. eq.</i>	<i>cc.</i>	<i>m. eq.</i>	<i>mgm.</i>	<i>mgm.</i>	<i>m. eq.</i>	<i>m. eq.</i>	
15-16	0	37.5	70.7	116	1510	58.2	148	418	13.5	21.5	Prodromal
16-17	0	38.0	71.3	116	1820	83.7	168	325	21.0	19.6	
17-18	0	37.8	71.5	116	1450	77.5	147	299	18.9	18.2	
18-19	+	38.0	72.0	116	1700	90.0	164	442	17.8	23.2	Attack
19-20	++	37.8	71.9	112	1800	103	182	524	18.4	28.2	
20-21	+	37.8	72.5	112	2120	135	191	630	18.5	27.9	
21-22	+	37.7	72.3	112	1850	112.3	186	512	19.3	24.9	
22-23	+	38.1	72.3	112	1680	111.1	190	422	18.9	22.8	
23-24	+	37.7	72.6	112	2020	111.2	202	535	21.0	26.8	
24-25	0	37.8	72.5	117	1950	90.2	179	520	19.4	24.3	Recovery
25-26	0	37.7	72.5	117	2000	101.9	182	551	19.9	22.3	
26-27	0	37.4	72.8	117	2050	110.8	182	393	19.1	25.7	
27-28	0	37.9	72.6	117	1680	95.6	181	390	17.9	22.9	
28-29	0	37.4	72.9	117	1770	94.6	151	445	17.6	19.7	

\* The following drugs were given: 19-20, pantopon 20 mgm., barbital 300 mgm.; 22-23, pantopon 20 mgm., colchicin 10 mgm.; 23-24, colchicin 14 mgm.

but the concentrations remained unchanged. The excretion of ammonia in the urine from K. H. was considerably less than in the urine from F. M. and two control subjects. This diminished excretion was probably related to the impaired kidney function. Daily blood samples for complete analyses were not drawn from this subject. In Table VI are given certain data for the whole blood and serum. Evidence of renal insufficiency may be deduced from the persistent elevation of the serum chloride concentration above 110 m.eq. and from the lowering of the serum  $\text{CO}_2$  content below 23 m.eq.

## DISCUSSION

This study of the quantitative changes associated with an attack of acute gout has yielded pertinent data concerning the disturbance of electrolyte balance in this disease. In one of the patients gout was uncomplicated by any other organic pathology; in the other patient, it was associated with renal damage. The analytical results from these patients were, in all respects, qualitatively similar.

The mass movement of *water* and *sodium chloride* before the appearance of symptoms was a constant observation during the 21 cycles studied on F. M. This water and electrolyte shift came later in the attacks studied on K. H. The diuresis from F. M. usually began 24 to 72 hours before the first symptoms of gout were complained of and before any elevation in body temperature

was observed. The maximum urinary output was observed directly before or on the day of maximum symptoms and was approximately twice the daily output observed during the recovery period. The return of the urinary volume to the normal level occurred over a period of several days which extended through the recovery period. It should be emphasized here that there was no medication during the prodromal period nor in most instances on the day of maximum diuresis. Any effect on the electrolyte and water balance from the drugs administered may be excluded with the exception of the last days of the attack period.

A diuresis before or early in an attack of gout was first observed by Scudamore (14) and has been amply confirmed by our data. Scudamore did little more than comment upon its occurrence, and he offered no explanation for the phenomenon. Clinically, a diuresis with a loss of body fluid and salt may be spontaneous or may result from experimental therapeutics. It is known that starvation, ketone acidosis, ammonium chloride acidosis, glycosuria, thyroxin and parathormone administration may be associated with an increased urinary output. There is little evidence that any of these factors is involved in the diuresis of acute gout.

The diuresis of adrenal insufficiency is not unlike the spontaneous diuresis of gout. In adrenal insufficiency (15) there is an increased excretion of Na with a loss of water secondary to the loss of base; likewise, in gout the loss of water ap-

TABLE VI  
*Experimental observations on blood and plasma on the patient K. H.*

Date	Whole blood						True plasma							
	Total $\text{CO}_2$	Oxygen capacity	Cell volume	Sugar	Creatinine	White blood cells	Bicarbonate	Chloride	Sodium	Total inorganic base	Phosphate	Non-protein nitrogen	Protein	Uric acid
April, 1934	m. eq. per liter	m. eq. per liter	per cent	mgm. per 100 cc.	mgm. per 100 cc.		m. eq. per liter	m. eq. per liter	m. eq. per liter	m. eq. per liter	m. eq. per liter	mgm. per 100 cc.	gms. per liter	mgm. per 100 cc.
15						6,250								
16	20.4	6.72	36.7	121	1.7	6,050	22.9	110.0	142.5	155.5	2.0	38.3	80.0	8.8
17						6,650							69.6	8.5
18						7,250						37.3	70.8	8.7
19						5,500								
20						5,750						34.8	66.4	9.1
21						6,250							68.3	8.9
22						7,150						38.1	63.0	8.3
23						6,750						34.9	68.5	8.8
24						6,050								8.2

pears to be secondary to the loss of Na and Cl. During the prodromal period, the period of increased excretion of these electrolytes, the urinary volume is increased, but during the recovery period there is not a concomitant decrease in volume with the significant decrease in the amount of base. While the introduction of adrenal insufficiency into this discussion presents an interesting analogy, it is not proposed to associate the pathogenesis of gout with a temporary deficiency of the cortical hormone.

A diuresis from a transient disturbance of purine metabolism has not been excluded. Certain purine bodies are known to possess a diuretic action, and one or more of these might be formed in sufficient amounts before the attack of gout to produce an increased urinary output. This explanation associates the diuresis intimately with the generally assumed pathogenesis of the disease.

The increase in body weight on the days of increased urinary output is almost paradoxical. On the second day of the prodromal period F. M. excreted more than 2 liters of urine, while his weight increased 0.5 kgm. Dr. J. P. Peters (16) has suggested that this may be a function of a difference in loss from insensible perspiration. Confirmation of this helpful suggestion is being sought at present.

The changes in the whole blood and serum that are associated with an attack of gout are of a lesser order of magnitude than might be anticipated from the data on urinary excretion. The decrease in concentration of serum Na was only 3 m.eq., while the urinary excretion of Na decreased from 125 m.eq. to 4 m.eq. The concentration of Cl in the serum decreased more than that of Na, and with this decrease there was an associated increase of the CO<sub>2</sub> content. These changes follow the loss of body fluid and presumably are not inherent in the attack of gout. The elevation of the serum K concentration that was observed during the prodromal period has not been noted in other diureses except that from adrenal insufficiency. There was no elevation of the serum Ca concentration during any attack, as was reported by Coates and Raiment (17). The blood sugar concentration was not followed through the cycle described here but was done during a subsequent cycle. The determinations were made because of a possible etiologic relation-

ship between gout and diabetes mellitus. The blood sugar concentration on the first day of symptoms was 87 mgm. per 100 cc. The concentration gradually increased until it reached 111 mgm. on the last day of maximum symptoms. During recovery the blood sugar concentration decreased to the level found preceding the attack.

An inquiry into the disturbance of the metabolism of uric acid was not the main object of this study; nevertheless certain data were obtained that are related to this subject. We did not confirm the observations of His (18) and Brugsch (19) of a decrease in the concentration of uric acid in the urine before an attack. The uric acid in the urine from the patient K. H. showed no significant change in the concentration during the 14-day cycle. An increased concentration in the urine from F. M. appeared late in the attack period. This may be explained by its occurrence on the day of minimum urinary output. The large daily excretion of uric acid by F. M. and the small excretion by K. H. presented a marked contrast. The average amount excreted by F. M. between attacks was about 800 mgm. daily and by K. H. it was 150 mgm. to 200 mgm. daily. The latter was about one-half the amount of uric acid excreted by two control subjects given a similar diet.

In most discussions of the pathogenesis of gout, the formation and destruction of uric acid have been considered to be of primary significance. The equilibrium of uric acid between blood, tissues and urine and the solubility of sodium urate in these media have been of secondary importance. From the observations of the solubility of sodium urate *in vitro*, there is little to suggest that its concentration is ever near the maximum solubility in blood or extracellular fluid (20). The concentration of uric acid in the urine, however, is frequently of a sufficient magnitude that its solubility may be influenced by the concentration of the other constituents (21). The reciprocal solubility between urates and sodium chloride as noted by Roberts (22) in aqueous solutions was not observed by us during the attacks studied.

This study of the water and mineral balance associated with attacks of acute gout presents the pathologic physiology of the disease from a different viewpoint than given heretofore. Our purpose is not to place less emphasis upon the disturbance of purine metabolism in gout; rather,



it is desired that gout should be considered a disease associated with a more comprehensive metabolic disturbance. The changes in the electrolyte balance in F. M., considered significant by us, were probably exaggerated because of the frequency of his attacks. The confirmation of these changes in a second patient with a complicating renal disease was most satisfactory. Various aspects of the pathogenesis of gout have been suggested by this work and either have been studied or are being studied at the present. The inciting of an acute attack by a diuretic and the prevention of the diuresis preceding an attack are included in this study. The influence of various minerals added to the diet has been investigated and will be reported later.

#### SUMMARY

1. Two patients have been studied during several attacks of acute gout while on a metabolic regime.

2. Changes in water and salt metabolism were observed as follows:

a. A diuresis begins before any clinical or subjective evidence of gout is manifest.

b. A negative sodium and chloride balance accompany this diuresis.

c. With the diuresis, there is also an increased excretion of potassium, calcium, ammonia, titratable acid, phosphate and urate.

3. The metabolic processes associated with acute gout are not adequately defined by a description of uric acid metabolism alone.

#### PROTOCOLS

*Case 1.* F. M., a single white man of 20, entered the Massachusetts General Hospital on August 8, 1932, complaining of polyarticular arthritis. The mother and father denied having had any arthritis or gout. At the age of 6 years, the patient had a pain in his left hip, a diagnosis of tuberculosis was made, and the leg was immobilized for 8 months. Subsequent recovery was satisfactory. When he was 12, he had an inflammation of several joints which persisted several days. During the next 4 years, a similar attack of short duration recurred yearly. Two years before his admission, he began to have from 3 to 5 attacks annually of polyarticular arthritis. These attacks subsided following administration of salicylates. He was fond of well-prepared food and alcoholic beverages.

On admission to the hospital, this patient was well developed and well nourished. The blood pressure was

150/85. Three subsequent determinations varied between 120/70 and 134/76. His chief complaint was pain in both hip joints. The physical examination revealed nothing unusual except tender hip joints. The eye grounds appeared to be normal. No tophi were found. A tentative diagnosis of atypical recurrent rheumatic fever was made. The laboratory studies were as follows: The red blood count was 5,250,000. The white blood cell count was 16,240. The urine at 5 examinations showed no albumin or sugar, and there were no casts or red cells in the centrifuged sediments. The whole blood uric acid concentration at 5 examinations varied between 4.7 mgm. per 100 cc. and 9.6 mgm. per 100 cc. A prostatic smear showed many white blood cells and red blood cells. The electrocardiogram was interpreted as normal. The x-rays of the right foot, both hands, both ankle joints, and the pelvis showed no variation from the normal. He was discharged one month after admission with a diagnosis of gout.

One year later he was re-admitted to the hospital for further study. About 3 months prior to this admission, he had noticed a nodule on the dorsal surface of the left index finger, and one month later a tophus appeared in the lobe of the right ear. He was in the hospital 2 months at this time and had 5 attacks of acute gout.

Two and one-half months later he was admitted to the metabolism ward of the hospital, where he remained about 7 months. On admission at this time he had several tophi that had not been observed previously. One tophus was over the left olecranon bursa, another was over the patellar bursa on the right, and there were several small nodules on the right patellar tendon. (The x-rays of his feet, knees, sacroiliac joints, hands, elbows and shoulders appeared normal. His basal metabolism, determined in the periods of freedom from attacks, varied between  $\pm 1$  per cent and  $\pm 6$  per cent. The whole blood uric acid varied between 6.5 mgm. and 9.7 mgm. per 100 cc. A urinary concentration test and a phenolsulphonephthalein excretion test showed a normal renal response. Crystals were removed from a tophus on the ear that were morphologically similar to those of sodium urate.

January 28, 1935, he was re-admitted to the metabolism ward. Additional tophi were observed over the extensor tendon of the right elbow, over the second phalangeal joint of the third finger of the right hand, and over the left phalangeal joint of the little finger of the right hand. The x-rays taken at that time were interpreted as follows: "The left shoulder joint shows faint shadows of calcification in the bursa. The left knee, right ankle, and both feet show degenerative changes about the joints, and there are definite punched-out areas of bone destruction involving the articular margins of the right great toe, the left second toe, and the left patella. The left knee joint space is definitely narrowed. These changes are consistent with gout. The elbows, sacroiliac joints, right knee, and right hand show nothing abnormal. No calcified blood vessel walls are seen."

Clinical diagnosis: gout.



*Case 2.* K. H., a widowed white woman of 44, entered the Massachusetts General Hospital on March 29, 1934, complaining of polyarthritis, nervousness, frontal headache and enlargement of her thyroid. Her mother had had rheumatism for many years which had been diagnosed as hypertrophic arthritis. The serum uric acid from her mother at one examination was 3.4 mgm. per 100 cc. The father of the patient had had mild joint disease which had been diagnosed gout. On one examination his serum uric acid concentration was 7.7 mgm. per 100 cc.

The patient had her first symptoms of joint disturbance at the age of 14. Since that time she had had several attacks yearly of low-grade arthritis. When she was 34 years old, a bilateral oophorectomy and a hysterectomy were performed. At the age of 38, she had pain and swelling in the right great toe which persisted for 4 weeks. One year later, she had her tonsils removed. Three years before admission, she had noticed a progressive enlargement of the thyroid. She had had no symptoms of hyperthyroidism, but complained of drowsiness, loss of hair and slowing of speech. One year before admission, she had an attack of right upper quadrant pain that suggested acute cholecystitis to the examining physician. She had had some frequency and dysuria.

On admission, she was well developed and nourished. A nodular goiter was observed, but there were no signs suggestive of myxedema. There were many lesions of acne rosacea on her face. The eye-grounds were normal. Examination of her joints showed tenderness and pain on motion of the mid-phalangeal joint of the right index finger and bony proliferation about the knee joints. The laboratory studies were as follows: The red blood count was 4,150,000; the white blood count was 11,000. The specific gravity of the urine varied between 1.007 and 1.013 in 3 samples collected hourly following 12 hours of abstinence from fluids and food. There was on several determinations a slight trace of albumin in the urine by the nitric acid test. The urinary sediment from 4 catheter specimens included numerous white cells, many bacteria and few red blood cells. No casts were seen. The excretion of 1 cc. of phenolsulphonephthalein injected intravenously varied, at the end of 15 minutes after injection, between 0.0 per cent and 8 per cent in 5 different tests. The whole blood uric acid concentration varied between 8.2 mgm. and 10.2 mgm. per 100 cc. The nonprotein nitrogen of the serum was 38 mgm. per 100 cc. The basal metabolic rate was —20 per cent and —17 per cent during the period of study reported at this time. The x-rays were interpreted as follows: "There is a rounded area of destruction at the base of the middle phalanx of the right hand. There is soft tissue swelling in this region, as well as about some of the corresponding bones of the other hand. There is narrowing of the joint spaces in these joints. There are some areas of subcartilage destruction in the corresponding joints of the second left finger. These changes might be produced by gout or chronic infectious arthritis." An x-ray taken

of the gallbladder region showed areas of increased density that suggested stones.

Clinical diagnosis: gout, cholelithiasis, cystitis, nodular goiter, secondary anemia and chronic nephritis (gouty kidneys?).

#### DIETS

Diet Number 4 was eaten by F. M. and contained the following amounts of foods: farina 25 grams, dates 25 grams, 40 per cent cream 50 grams, grapefruit sections 200 grams, orange sections 200 grams, white bread 85 grams, salt-free butter 50 grams, jelly 10 grams, milk 350 cc., macaroni 25 grams, American cheese 25 grams, corn niblets 100 grams, whole egg 100 grams, potato 100 grams, prunes 50 grams, honey 35 grams, tomato juice 25 grams, penuche containing brown sugar 90 grams, and 40 per cent cream 20 grams, sugar 25 grams, sodium chloride 1.45 grams.

Diet Number 6 was eaten by K. H. and contained the following amounts of foods: corn flakes 40 grams, dates 25 grams, 40 per cent cream 100 grams, grapefruit sections 200 grams, orange sections 200 grams, white bread 85 grams, salt-free butter 50 grams, jelly 10 grams, milk 350 cc., macaroni 25 grams, American cheese 25 grams, corn niblets 100 grams, whole egg 100 grams, potato 100 grams, prunes 50 grams, penuche containing brown sugar 90 grams, and 40 per cent cream 20 grams, sugar 52 grams, sodium chloride 1.45 grams.

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# A STUDY OF THE LEUKOCYTOSIS PRODUCED IN MAN BY ARTIFICIAL FEVER<sup>1</sup>

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The use of artificial fever in the treatment of various diseases offers an opportunity to observe some of the specific effects brought about in the human body by changes in temperature alone. Variations occurring in the number and kind of cells in the circulating blood may be studied during fever artificially produced by radiant energy with the elimination, in the main, of some of the complicating factors (foreign proteins, toxins, etc.) which may play a part in the spontaneous rises in temperature resulting from acute disease. A knowledge of the peculiar influence of fever upon the white blood corpuscles is important in evaluating the rôle of infection in differential diagnosis, and may change somewhat our concept of a leukocytosis in the presence of a febrile disease. The development of a non-specific leukocytosis with artificial fever emphasizes, perhaps, the retarding mechanism, or negative stimulus, in such diseases as typhoid fever, measles, influenza, etc., which are characterized by a leukopenia.

The method (3) of maintaining the body temperature constantly and accurately at a certain fever level for periods of hours, presents an artificial set-up particularly suitable for study of the effect of fever upon the blood cells. The use of any of the several electrical methods to elevate and maintain the patient's temperature is followed by the same relative changes and does not influence the results. This is borne out in our own clinic with the use of diathermy, radiothermy, and radiant energy, and by the reports of others (2).

In a study of patients treated with the radiotherm, Hinsie and Carpenter (7) found a slight reduction in red blood cell count and hemoglobin during a series of treatments. There was also an increase in polymorphonuclears and a relative decrease in lymphocytes. Hinsie and Blalock (6) reported an increase of about 75 per cent in leukocyte count as a result of artificial fever pro-

duced by the radiotherm and carried on for 7 hours by wrapping the patients in blankets. This rise usually reached a maximum at the end of the 9th hour, and regained its normal level at the end of about 20 hours. They found the rise to be less pronounced over a course of 20 treatments. The leukocytosis was characterized by an increase in the percentage of polymorphonuclears at the expense, chiefly, of the lymphocytes. They also found an increase in the non-filamentous forms. Tenney (15) reported similar findings.

In the use of the radiotherm, Bierman and Fishberg (2) found an increase of white blood cells during the period of temperature elevation. The percentage of polymorphonuclears was increased and that of the lymphocytes was correspondingly lowered. He also found an increase in the number of red blood cells, and in many instances a marked increase in the immature forms of red blood cells.

A group of patients, subjected to diathermy, was studied by Perkins (14). All of these patients were suffering from dementia paralytica. The temperature was raised to 105° F. and then allowed to drop to normal as rapidly as possible. Patients were given ten such treatments, five per week for two weeks. He found a leukocytosis in this group which was not particularly affected by the first few treatments given, but was decidedly increased by the time the entire series of ten treatments had been completed, and this increase persisted for at least three days after the completion of therapy.

In patients treated with balneotherapy, Neymann (13) found an increase in white blood cells, especially of polymorphonuclears, directly after the bath. In patients treated with diathermy, he reported an increase of one million red blood cells per cu. mm. and a slight relative increase of polymorphonuclears and eosinophiles, and a corresponding decrease of small and large monocytes. These values returned to a normal level 24 to 48 hours after treatment.

In all of these studies the body temperature varied considerably over the febrile period.

Bunker (4) reviewed the literature on the parenteral introduction of various protein substances into the organism. Almost always the injection of these substances was followed at a brief interval by a definite leukopenia, which was not infrequently succeeded by a leukocytosis. In patients inoculated with tertian malaria, this observer found that there was a definite reduction in the number of leukocytes in the peripheral blood in 30 out of 33 instances, with a subsequent leukocytosis of somewhat moderate degree in 27 out of 35. The reduction

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had its onset at about the time of the chill, reaching its maximum during the latter part of the ascent or at the apex of the temperature curve. (The maximum degree of leukocytosis usually followed by 2 to 4 hours the time of maximum leukopenia, and appeared near the summit of the temperature curve, or more usually during its gradual descent.

Knudson and Schaible (9), in a study of dogs kept in an ultra-high frequency field, found a considerable increase in the number of red blood cells and the hemoglobin, and a marked increase in immature forms of red cells. There was also a marked increase in the total white blood cells due to an absolute and relative increase in the polymorphonuclears. The lymphocytes and eosinophiles were usually relatively markedly decreased. The changes in monocytes were less marked and less constant. A rather similar change was found by Lawrence, et al. (8, 10) following the injection intravenously and intraperitoneally of sodium bicarbonate or ammonium chloride or infection of the peritoneal cavity with *B. coli* or the injection of killed cultures of *B. coli* and diphtheria toxin in the guinea pig. Similar changes have been observed in dog and man. A postoperative neutrophilic leukocytosis was found by the same workers in obstructive jaundice experimentally produced in dogs while the response of the lymphoid series was inconstant.

Murphy and Sturm (11) exposed mice, rats and guinea pigs to dry heat from 55° to 60° C. for five minutes, and noted an immediate fall in white blood cells. The polymorphonuclears recovered slowly, and required several weeks to regain the normal number. The lymphocytes rose rapidly after the initial fall, and continued to rise for two or three weeks, often 200 per cent to 300 per cent above normal.

Caillet and Simonds (5) heated mice repeatedly with air at 60° C. for five minutes at intervals of 10 days. (This resulted in a temporary lymphopenia followed by an increasing lymphocytosis up to about the 40th day, or the third or fourth heating. After this time, each successive heating became less and less effective, until after about 200 days, or 12 to 13 heatings, the total leukocyte count and the per cent of lymphocytes returned to, or below, normal and showed little or no effect from further heating. No record of the temperature of the animals is given, and these experiments cannot be compared directly because the animals were totally enclosed in the hot air chamber. In brief, identical changes may be brought about by the circulating blood cells by several methods which do not seem at all related.

#### METHOD AND MATERIAL

In order to determine the changes occurring in the number of blood cells during artificial fever treatments, in which the patients' temperature was elevated and maintained at a constant level for a definite period by radiant energy, 10 patients were studied during a total of 11 treatments. There were 7 treatments for the gonococcus infection;

one patient in this group was also suffering from latent syphilis and chronic infectious arthritis. The diagnoses in the remaining 4 cases were syphilis of the central nervous system, chronic infectious arthritis, brain tumor, and carcinomatosis. All observations of temperature during treatment were made by means of a resistance thermometer placed in the rectum of the patient.

In the patient suffering from infectious arthritis alone, the rectal temperature was elevated for 4½ hours at 40.5° C. There were 9 treatments in which the temperature of the body was raised to 41.5° C. and 41.6° C. and maintained between 5 hours and 5½ hours, exactly at this temperature. Included in this group were the cases with the gonococcus infection, the case of brain tumor, and that of syphilis of the central nervous system. The body temperature of the patient with carcinomatosis was raised to 41.6° C. and maintained exactly at that level for 21 hours. All patients were afebrile before and after treatment.

In practically every case the red blood cell count, hemoglobin, white blood cell count, and differential count, including the Schilling hemogram made from cover-slip blood smears stained with Wright's stain were determined before treatment and every hour, starting at that time at which the temperature had reached the desired height, and was being maintained at a constant level. These determinations were continued for several hours after the termination of the fever (during which time the patient's temperature was normal). The blood studies were then followed at intervals until the patient was discharged from the hospital. All of the blood cell counts were done under constant conditions by one of us (P. C.), using the same pipettes, counting chamber, and a calibrated hemoglobin standard (100 per cent equals 13.8 grams hemoglobin per 100 cc.). The counts were made from blood taken from the ear.

#### RESULTS

In all but two cases the maximum increase in white blood cells was more than 100 per cent of the original number. In the case of the patient with the lower fever temperature (40.5° C.) the maximum increase was only 49.1 per cent and 36.6 per cent in one of the patients with the tem-

perature held at 41.6° C. for 5 hours. The maximum increase was over 200 per cent in two cases (temperature 41.6° C. for 5 hours), and reached 315 per cent in the patient with the prolonged fever (41.6° C. for 21 hours).

The maximum increase in white blood cells appeared at different times, and the time of the drop to the normal figures varied. In three of the cases the maximum increase occurred at the end of the period of fever. These three cases had a fever of 41.6° C. of 5 hours' duration. In two of the cases the maximum increase occurred at the third hour of the fever. In two more cases the maximum increase was noted 2 hours after the termination of the five-hour fever. The peak of the leukocytosis was noted at the 4th hour of the fever in two cases. In the case with the lower fever (40.5° C. for 4½ hours), it appeared one hour after the termination of the fever. In the case in which the higher temperature (41.6° C.) was maintained for 21 hours, the maximum increase was noted at the 10th hour. In the latter case, blood counts were made at less frequent intervals.

The return to normal or the previous level varied. One case had a white blood corpuscle count below 10,000 per cu. mm. within three hours following the termination of the fever; another within 6½ hours. The count of the patient with the low fever reverted to normal in 6 hours. In the case of the patient whose maximum increase was only 36.6 per cent, the white blood cell count returned to the pre-treatment level in 2 hours. The original white blood cell count in this case was 11,600 per cu. mm. In six cases the counts were slightly elevated 6 to 8 hours after the treatment was terminated. In five cases the counts were normal by the following day. In one patient it was still slightly elevated. The white blood cell count of the patient who received the very long fever was still markedly elevated the day following the termination of the fever, although the temperature at the time was normal. The cell counts obtained from this patient on the third day were normal.

Along with the increase in the total number of white blood cells, there was a marked relative and absolute increase of polymorphonuclears in all cases. Before treatment there was no case in which the percentage of these cells was above 72

per cent. As a result of the fever, the polymorphonuclears increased in every case, varying from 84 per cent to 93 per cent. Likewise, the total number of the polymorphonuclears increased. Before treatment the maximum number of these cells in any case was 8,400 per cu. mm. The fever caused the total number of these cells to vary between 11,760 and 23,200 per cu. mm.

There was a substantial increase in the relative and absolute numbers of immature forms of polymorphonuclears as a result of the fever in more than half of the cases. The pre-febrile level varied from 0 to 7.5 per cent. In five of the cases, including the patient with the short fever, they remained below 6 per cent, while the rest varied from 12 per cent to 24 per cent during or following the fever treatment.

The decrease in relative and absolute numbers of lymphocytes was as marked as the increase in polymorphonuclears. Before the fever the percentage of lymphocytes varied from 21 per cent to 42 per cent. The low point in lymphocytes in the individual cases varied from 1 per cent to 10 per cent. The fall in absolute numbers was not as great as the difference in percentages might indicate, due to the great increase in the total white blood cell count. However, in practically every case the number of lymphocytes was less than half of the original, and in one case was ⅓ of the original.

There was a tendency for the monocytes to increase, both in absolute and relative numbers. The changes in basophiles and eosinophiles were not significant.

In general, there was only a slight rise in hemoglobin (0 to 10 per cent). In two cases, however, it rose to 20 per cent above the level obtained before treatment. One of these was the case treated for 21 hours. There was a corresponding rise in the red blood cell counts. The maximum increase usually appeared toward the end of the treatment, or shortly after the treatment was terminated.

In the blood of the patient treated for 21 hours, normoblasts were found from the 10th hour to the 21st hour of the fever. One to three such cells were found while 200 white blood cells were being counted. In none of the other patients were normoblasts found.

Four of the cases are reported in some detail to show the character of the changes noted.

The lowest elevation of temperature studied was in a patient (F. M.), weighing 96 kgm., suffering from chronic infectious arthritis of three years' duration, with a normal temperature (36.9° C. rectal) and an initial white blood cell count of 9,400 per cu. mm. The rectal temperature was elevated to 40.5° C. by means of radiant energy within 70 minutes and maintained at 40.5°

C. for 4½ hours by means of radiant energy. The temperature was then lowered (by blowing air upon the patient's skin) in 1½ hours to 37.0° C., and after one more hour it remained normal (36.5° to 36.7° C.). Complete blood studies were made at hourly intervals between 9:00 a.m. to 8:40 p.m., except the last observation during the height of the fever, which interval was extended 30 minutes (1½-hour interval) to include the end of the febrile period. A complete blood

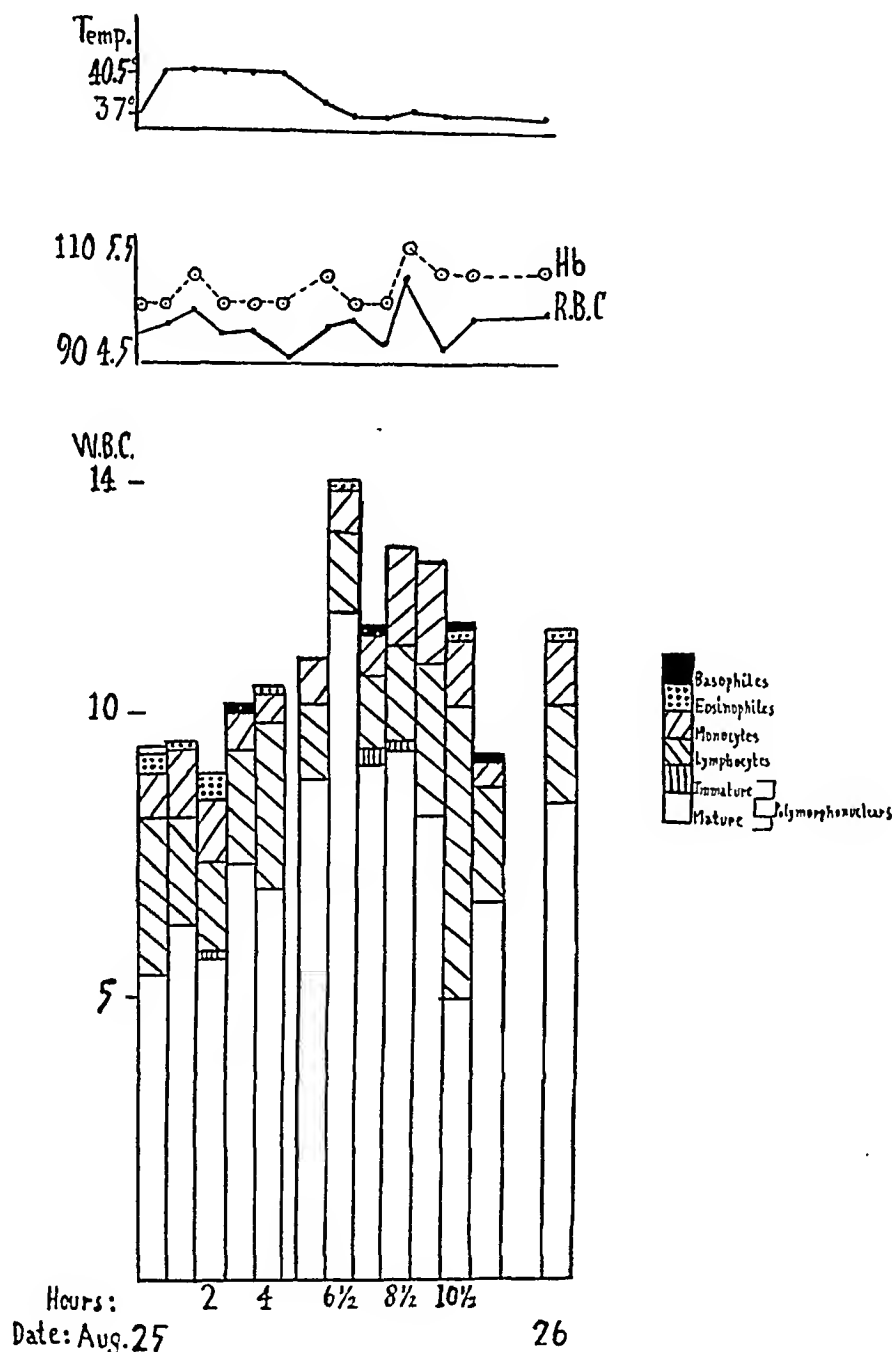


FIG. 1. (F. M.)

Temperature is expressed in °C., R.B.C. in millions, Hb in per cent, and W.B.C. in thousands. 5 hours of fever at 41.5° C.

study was done at 9:30 a.m. the following morning. Barbitol and chloral hydrate were used for narcosis. The patient was given all the fluids he would drink during and after the treatment. The maximum fluctuation of the hemoglobin was no greater than 10 per cent, and this occurred 4 hours after the end of the febrile period and later than the peak of the leukocytosis. The maximum elevation of white blood cells occurred one hour after the termination of the fever (temperature of 38.0° C.). The immature polymorphonuclear leukocytes did not increase above the normal percentage during the rise in total white blood cell count to 14,00 per cu. mm. There was a slow steady fall in total white blood cell count after this. The chart and table give the complete data (Figure 1, Table I).

Another patient (M. T.) received two artificial fever treatments of five hours each at 41.6° C., 16 days apart. This was an 18-year old girl, who was suffering from acute gonorrheal urethritis and salpingitis. The white blood cell count at the time the patient was placed in the radiant

energy cabinet for her first treatment was 9,600 per cu. mm. Her rectal temperature was 37.0° C. A period of 100 minutes elapsed before the patient's temperature reached the desired level. Blood studies were made every hour from this time throughout the febrile period, and for 6 hours following this period. The maximum increase in white blood cell count noted in this case occurred at the 3d hour of fever and reached 19,800 per cu. mm. It fell gradually from this time on and when the last count was made that evening it was 11,800 per cu. mm., i.e. slightly elevated, although the temperature then was 36.5° C. The following morning the white blood cell count was 9,000 per cu. mm. This patient's hemoglobin rose once and only 5 per cent. This increase appeared at the time the temperature reached 41.5° C., and it remained at the previous level throughout the rest of the period. The mature and immature polymorphonuclears increased in relative and absolute numbers, while the lymphocytes decreased.

TABLE I

*Correlation of total number of leukocytes with absolute values before and at the maximum rise.*

Patient	Diagnosis	Fever		Initial W.B.C.	Maxi- mum W.B.C.	Initial poly.	Maxi- mum poly.	Initial immature poly.	Maxi- mum immature poly.	Initial lymph.	Mini- mum lymph.	Initial Hb	Maxi- mum Hb	Initial R.B.C.	Maxi- mum R.B.C.
		Dur- ation	Temper- ature												
F. L....	Chronic gonococcal cervicitis and urethritis.	hours	° C.	per c. mm.	per c. mm.	per c. mm.	per c. mm.	per c. mm.	per c. mm.	per c. mm.	per c. mm.	per cent.	per cent.	millions	millions
		5	41.5	4,650	14,750	2700	13,720	93	717	1350	442	75	85	3.29	3.92
M. T. (1)	Acute gonococcal cervicitis.	5	41.6	9,600	19,800	6720	18,600	192	2410	2020	193	70	75	3.70	4.25
M. T. (2)	Urethritis and salpingitis.	5	41.6	7,000	24,000	3640	18,750	70	1200	2520	1233	70	70	3.83	4.66
H. LaF..	Acute gonococcal cervicitis, urethritis and salpingitis.	5	41.6	7,950	22,900	5100	19,700	795	5030	2530	597	90	100	4.10	4.89
B. S....	Acute gonococcal con- junctivitis, cervicitis and urethritis.	5	41.6	11,650	27,000	7350	23,200	815.5	5565	3730	1265	95	110	3.93	4.79
P. S....	Chronic gonococcal cervicitis and salpingitis.	5.7	41.6	7,100	21,400	3403	19,050	142	2565	2932	323	85	100	3.93	4.23
G. S....	Chronic prostatic urethritis, epidy- dimitis and chronic infectious arthritis.	5.3	41.6	8,100	17,750	5731	14,853	81	2976	1853	334	85	100	4.23	5.23
C. A....	Tabo-paresis.	5	41.6	11,600	15,850	8352	13,217	232	777.5	2204	622	110	115	5.59	5.63
C. T....	Brain tumor, unverified.	5	41.6	5,100	18,950	2910	17,050	0	565.5	1633	753	100	120	4.90	5.55
F. M....	Chronic infectious arthritis.	4.5	40.5	9,400	19,000	5452	11,760	0	230	2725	1400	100	110	4.75	5.23
B. W....	Cardiomyositis.	21	41.6	4,850	20,150	2940	18,120	239.5	4540	1390	893	70	90	3.79	4.15



The patient still had gram-negative diplococci in urethral smears and was given a second artificial fever treatment 16 days later. The leukocyte count before the second treatment was 7,000 per cu. mm. (rectal temperature 37.3° C.). One hundred minutes elapsed before the temperature reached the desired level (41.5° C.) and blood

counts were made at hourly intervals as in the first treatment. The leukocyte count reached 24,000 per cu. mm., which occurred 2 hours after the fever was terminated. The patient's temperature at this time was 37.6° C. Seven hours after the termination of the fever the white blood cell count was still elevated, 14,800 per cu. mm., with

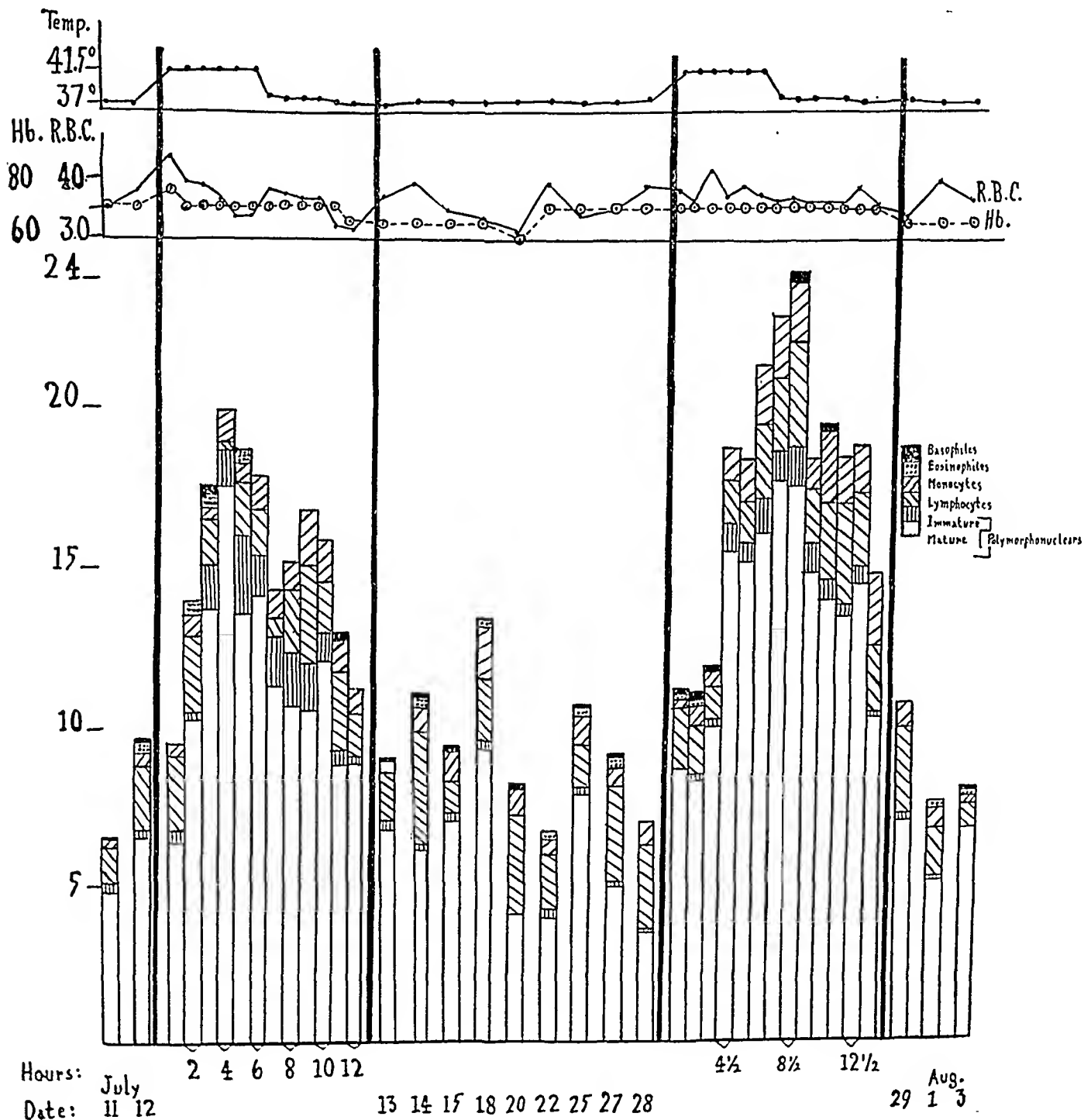


FIG. 2. (M. T.) TWO FIVE-HOUR FEVERS AT 41.5° C. TEN DAYS APART.

Temperature is expressed in °C., R.B.C. in millions, Hb in per cent, and W.B.C. in thousands. The intervals between the longitudinal, heavy lines represent hourly determinations on the days of the fever treatment. See legend of Figure 1.

a temperature of 37.3° C. The following morning the white blood cell count was 10,750 per cu. mm. The hemoglobin during this treatment did not vary at all. Similar changes in polymorphonuclears and in lymphocytes were noted as in the first treatment, except that this time the immature polymorphonuclears did not go above 5 per cent of the total white blood cells. However, there was a substantial increase in absolute numbers of these cells. This patient also received barbitol and chloral hydrate as a narcotic at the beginning of the treatments (Figure 2, Table II).

The longest fever period in this series was one of 21 hours at 41.6° C. This patient (B. W.) was suffering from carcinomatosis and this was her fourth fever treatment. She had had one treatment of 6 hours, one of 7 hours, and one of 13 hours at 41.6° C. in the two month period before this treatment. Barbitol, sodium bromide and chloral hydrate were used for narcosis. Her white blood cell count was 4,850 per cu. mm. three days before this long artificial fever treatment was given. During the febrile period blood counts were made at approximately 5 hour

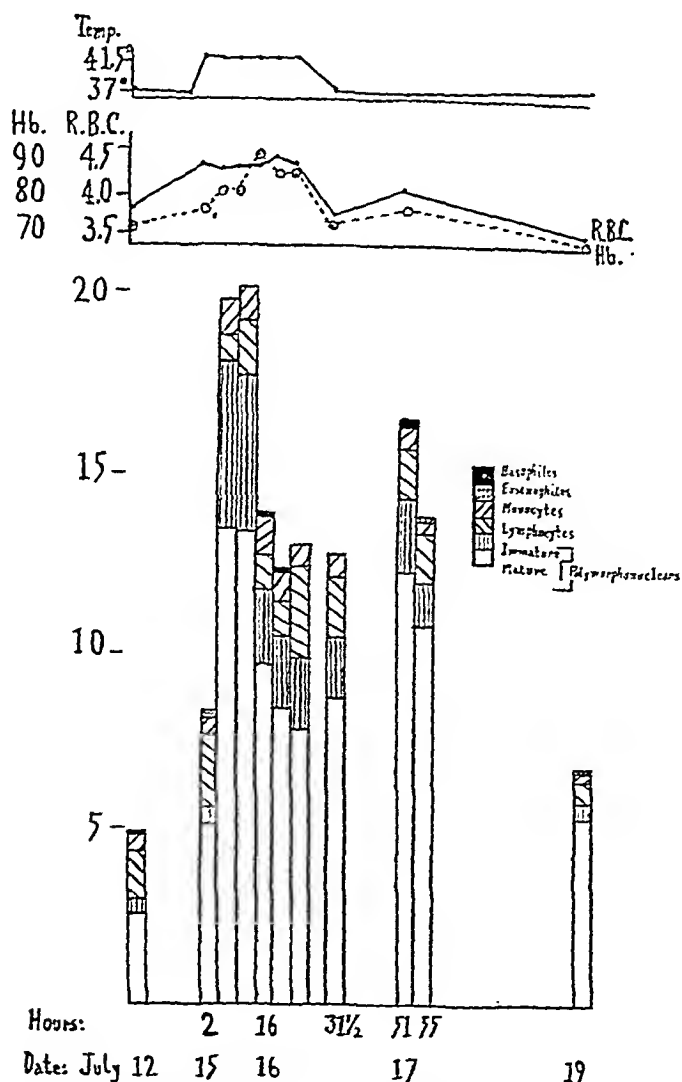


FIG. 3. (B. W.) TWENTY-ONE HOURS OF FEVER AT 41.5° C.

Temperature is expressed in °C., R.B.C. in millions, Hb in per cent, and W.B.C. in thousands.

intervals. The highest white blood cell count obtained in this case was 20,150 per cu. mm. at the 10th hour of the fever. A leukocytosis of 13,850 per cu. mm. was still present 32 hours following the termination of the fever, although there was no elevation of temperature. The third day following the termination of the fever the count was

normal. The absolute and relative increase in mature and immature polymorphonuclears was also noted in this case, as well as the absolute and relative decrease in lymphocytes. The hemoglobin increased 20 per cent. The patient was given all the fluids she would take. The maximum increase in white blood cell count was noted

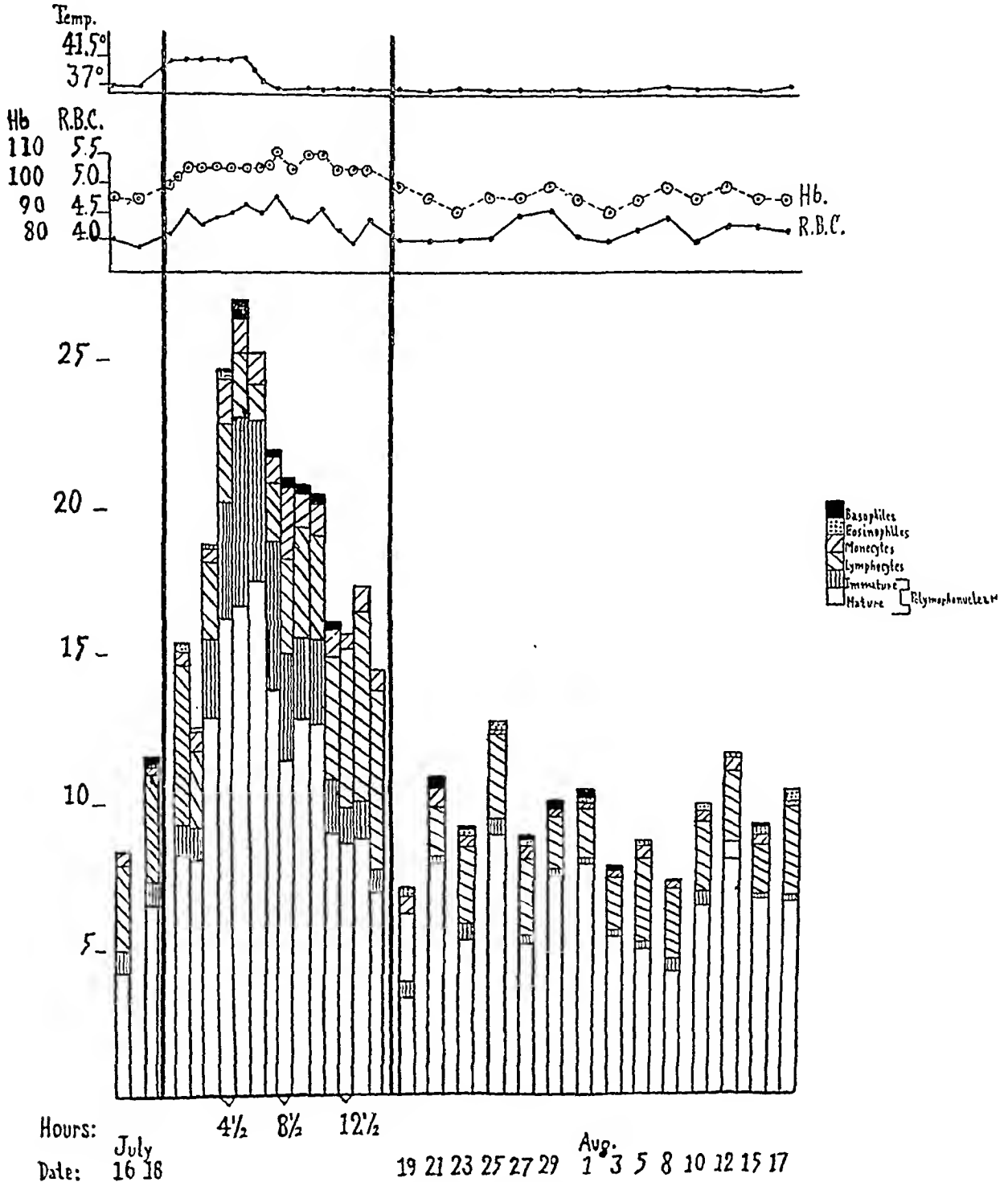


FIG. 4. (B. S.)

Temperature is expressed in °C., R.B.C. in millions, Hb in per cent, and W.B.C. in thousands. The intervals between the longitudinal, heavy lines represent hourly determinations on the day of the fever treatment.

at the 14th hour. Normoblasts were found in the smear from the 10th to the 21st hour of the fever (Figure 3, Table III).

The greatest leukocytosis occurred in a patient (B. S.) who was suffering from gonorrheal conjunctivitis and gonococcus infection of the lower birth canal. Barbitol, sodium bromide and chloral hydrate were used for narcosis. The leukocyte count on the morning of her fever treatment was 11,650 per cu. mm. The rectal temperature was 37.2° C. at this time. The body temperature was raised to 41.6° C. in 1½ hours and maintained at this level for 5 hours. Blood counts were made every hour until the 8th hour after the fever. The white blood cells increased to a maximum of 27,000 per cu. mm. at the 4th hour of the treatment. The leukocyte count decreased gradually to 14,600 per cu. mm. at the end of the 8th hour, with a rectal temperature of 36.8° C., following the treatment. On the following morning the count was 7,100 per cu. mm. The increased relative and absolute numbers of polymorphonuclears were also noted in this case. The immature polymorphonuclears increased from 7 per cent to 24 per cent. There was also a fall in the relative and absolute numbers of lymphocytes during the fever. The hemoglobin was elevated 15 per cent at the 2nd hour after the treatment. The blood counts in this patient were followed for a month following the treatment, during which time there was no evidence of the gonorrheal infection. There were no marked changes in the counts during this time (Figure 4, Table IV).

Table V summarizes the results in all the cases studied.

#### DISCUSSION

Any strict comparison of the individual cases upon the basis of percentage and relative numbers is not possible because of the small series studied, but several general conclusions may be drawn, since our observations are in keeping with the general findings of others who have used other means (radiant energy) for producing artificial fever.

Although the nearly mature polymorphonuclear leukocytes did not increase in every case, it is interesting that these immature cells appeared in large numbers during the peak of the leukocytic

response and then decreased as the number of leukocytes decreased.

The fall of from 28.5 per cent to 6.5 per cent in the lymphocytes in the patient receiving the 21 hour fever treatment (41.6° C.), as well as the fall in lymphocytes in the other cases, cannot be explained. Whether these cells are stored somewhere in the body or take some specific part in attacking the pathological process present or are destroyed by the febrile reaction is not clear. The evidence in the experimental data published (Nakahara) (12) is not sufficient, in our opinion, to warrant the assumption that they are destroyed.

The slight amount of concentration of the blood, as evidenced by the rise in hemoglobin percentage and the red blood cell count, is probably not responsible for the leukocytosis, which is far out of proportion to the increase in hemoglobin, although many complicated factors are involved such as the increased demand for oxygen during the fever. Barbour (1) has stated that blood is first diluted and then concentrated whenever there is a considerable rise in temperature. Every precaution was taken to prevent dehydration and, therefore, concentration of the blood. In some of the patients the maximum increase in hemoglobin percentage occurred after the treatment was finished. At the termination of the treatment, during the 60 to 80 minutes of the cooling-off process, the fluid intake is usually 500 to 1000 cc., which would tend to cause dilution rather than concentration of the blood. Later as the body tissues store fluids some such change might be expected though it was not observed. Also, the change in the distribution among the types of the white blood cells is another point against the leukocytosis being a result of the concentration of the blood alone.

This finding, namely, a leukocytosis during a prolonged, maintained artificial fever, suggests that an elevation of the white blood cell count in a patient with a spontaneous fever (due to infection) may in certain cases be an index of the fever rather than of the extent of the infection. A leukocytosis with a low fever, or without fever, may be of greater clinical significance. It should be emphasized that many factors in addition to the fever may be responsible for the leukocytosis; in fact, as suggested by Lawrence et al. (10), tissue autolysates may be the main stimulus. Dur-

ing fever the production of these may be increased in sites of inflammation. The balance between the circulating and stored mature cells is probably a critical one and one which artificial fever, as well as spontaneous febrile reaction, may readily upset. Upon the subsidence of the fever the balance is rapidly restored to its previous level. Some of the cells of the myeloid and erythrocyte series that are nearly mature are also capable of appearing and disappearing (i.e., being stored as immature cells if they do not mature in the blood) in response to a febrile reaction.

An increase in pulse rate (of 100 to 200 per cent) and presumably an increase in circulation rate might cause the cells to be washed out of storage sites into the circulation, but in some patients the peak of the leukocytosis occurred after the treatment, when the pulse rate, blood pressure and presumably the circulation rate had returned to normal or nearly normal. The appearance of these immature cells would suggest the mobilization of all the available cells (mature and nearly mature, both leukocytes and erythrocytes) into the circulating blood. The increase may represent the total in reserve, or number available, at any given short period of time (hours) and before the stimulus calling them out has had time to activate the maturation or formation of a large number of new cells in the marrow or myeloid structures, such as would occur over a longer period of time from the reaction to an infectious process in the body. There is no evidence pointing to the place of storage of these cells after they are called forth, nor do we need to suppose that they are destroyed after the supposed need for them is past.

The patients studied were as nearly normal as any of those receiving treatment in our clinic, and the constancy of the reaction would seem to indicate that the qualitative nature of the changes observed are significant of the changes brought about primarily by the elevation of the temperature. As suggested above, however, the destruction of tissue in the pathological conditions treated may influence the situation somewhat; in this connection it is notable that the case of acute gonorrheal infection showed the greatest leukocytosis.

The number and kinds of observations may seem few, but they represent all that the individual patient would tolerate during the treat-

ment by artificial fever. In the stage of semi-delirium at 41.5° C. the patients may be upset easily by any slight irritation. In some patients it was very difficult at times to obtain accurately filled pipettes because of the antagonism of the patients to the procedure, making it necessary to repeat this procedure many times.

#### SUMMARY

1. The cytological variations in the blood resulting from prolonged artificial fever, produced by radiant energy, were studied in 10 afebrile patients (only one with acute disease) during a total of eleven treatments.

2. A leukocytosis was found in every case. The maximum change, the onset, duration and the extent of this change, in relation to the fever period varied from patient to patient.

3. A relative and absolute increase of polymorphonuclear leukocytes was observed during, or immediately following, the febrile period.

4. There was a substantial relative and absolute increase in immature polymorphonuclear leukocytes in 6 of the 11 cases.

5. There was a relative and absolute decrease in lymphocytes in all cases.

6. There was a slight rise in the red blood cell count and hemoglobin during or immediately following the period of fever. Immature red blood cells were found in a single case, which also exhibited the greatest increase (20 per cent) in hemoglobin (during a fever of 41.6° C. for 21 hours).

7. These observations suggest a mobilization into the circulation of available and nearly mature cells of the myeloid and erythrocytic series as a result of the fever, while the cells of the lymphoid series decrease (storage ?) during this period.

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# ON AN URTICARIAL RESPONSE TO LIGHT AND ITS PHOTOPHYSIOLOGY<sup>1</sup>

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The opportunity has recently been afforded to study a case of abnormal sensitivity to sunlight which fits the description of *urticaria solaris* given by Duke (1923) and others. Certain observations have been made which shed considerable light upon the photophysiology and photopathology of skin and will be described herein. The clinical findings will be reviewed elsewhere in the near future and it will suffice here to say that the patient has appeared at the time of our observations, and subsequently, to be completely normal, except in the fact that a few minutes' exposure to sunlight produces an immediate and severe erythema and edema accompanied by itching. The type of reaction involved appears to be sharply differentiated from the normal reactions of the skin to light, and in the following discussion we will follow the plan of contrasting the two phenomena.

*General description.* When a portion of the skin of this individual is exposed to sunlight for a few minutes, a marked erythema and edema appear. With short exposures, say two minutes, the erythema may not appear until a few minutes after the cessation of the irradiation. This erythema is discreetly limited to the area exposed. After a short time edema appears, likewise restricted to the exposed area, and still later an erythema develops surrounding and spreading outward from the edematous area. Figure 1 shows the appearance of the edema and erythema following an irradiation of three minutes. A very short exposure may result in an erythema only, without edema. In all cases, even the most severe, both edema and erythema disappear in the course of a few hours, leaving no discoloration of the exposed skin nor any other trace of the occurrence.

The resemblance to the "triple response" described by Lewis (1927) is striking. He clearly

demonstrated the similarity of such reactions to that produced by histamine pricks and formulated the hypothesis that a histamine-like "H" substance is released as the result of cell damage and is responsible for the "triple response." We are immediately led to the hypothesis that in the case herein described, a photochemical reaction occurs in the skin which results in the release of "H" substance.

This type of response differs very markedly from the reaction of normal skin to ultraviolet light. For example, immediately following exposure of a normal skin to the radiation from a quartz mercury arc, no erythema is observable other than a slight one produced by heat. After a period of an hour or more, an erythema appears which may persist for a day or more, and pigmentation begins to appear before this erythema has subsided; edema does not ordinarily occur as a result of moderate doses. While Lewis (1927) believed that the delayed erythemic response following ultraviolet irradiation is an example of the "triple response," resulting from the elaboration of "H" substance, Krogh (1929) contended that it is probably dependent upon the production of another less diffusible substance. At any rate, the difference in the rate of appearance of the abnormal response and that of the normal, a matter of minutes as against hours, indicates a distinct difference in the two processes even if both be the result of production of "H" substance.

*The active wave lengths.* In Figure 2A is shown a curve representing the relative effectiveness of the wave lengths which elicit the erythema of normal skin.<sup>2</sup> It will be seen that the erythema-producing radiations are limited to those of wave lengths less than 3200 Å, and that there is a sharp band of active wave lengths having its maximum at 2970 Å. Proceeding toward the

<sup>1</sup> This investigation was assisted by a grant from the Board of Research of the University of California.

<sup>2</sup> The curve is taken from that of Lukiesh, Holladay and Taylor (1930).



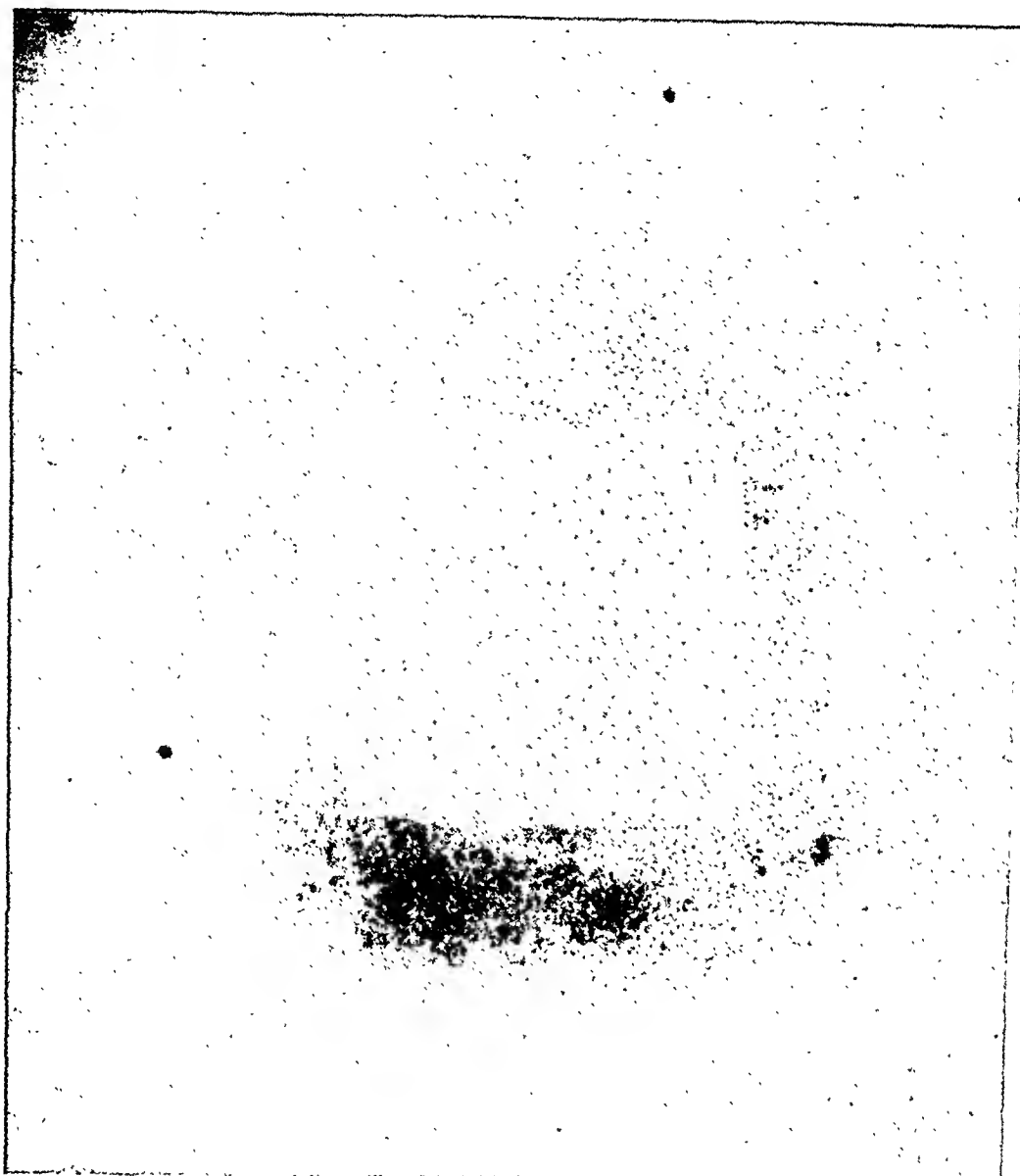


FIG. 1. Urticarial reaction of skin of back following 3 minutes of exposure to sunlight. The square area of edema corresponds to the area exposed to the sun's rays. The spreading area of erythema around the edematous region is clearly shown.

short wave lengths of the spectrum, a minimum occurs at about  $2800 \text{ \AA}$  followed by a second increase in effectiveness. Three groups of workers agree on the position of the maximum at  $2970 \text{ \AA}$ , but there is not complete agreement as to the values for the shorter wave lengths. In general there seems to be some disagreement as to whether pigment production corresponds to exactly the same wave lengths as erythema production, but certainly the agreement is good in the region of  $2970 \text{ \AA}$ .<sup>2</sup>

From the curve it is reasonable to assume that the maximum at  $2970 \text{ \AA}$  represents an absorption

band of some compound which when activated brings about reactions which end in the production of erythema and pigmentation. Obviously, an abnormal sensitivity to sunlight might represent an enhanced activity of this normal erythema-producing mechanism, but in this case the wave lengths active in bringing it about *must* be the same as those which elicit the normal reaction.

For this reason it is important to know the wave lengths which are active in bringing about abnormal skin effects, and we have made a sufficient number of observations to determine effectively the wave lengths which bring about the abnormal edema and erythema in our patient. Table I shows the results of various exposures to

<sup>2</sup> For a discussion of this see Laurens (1933).

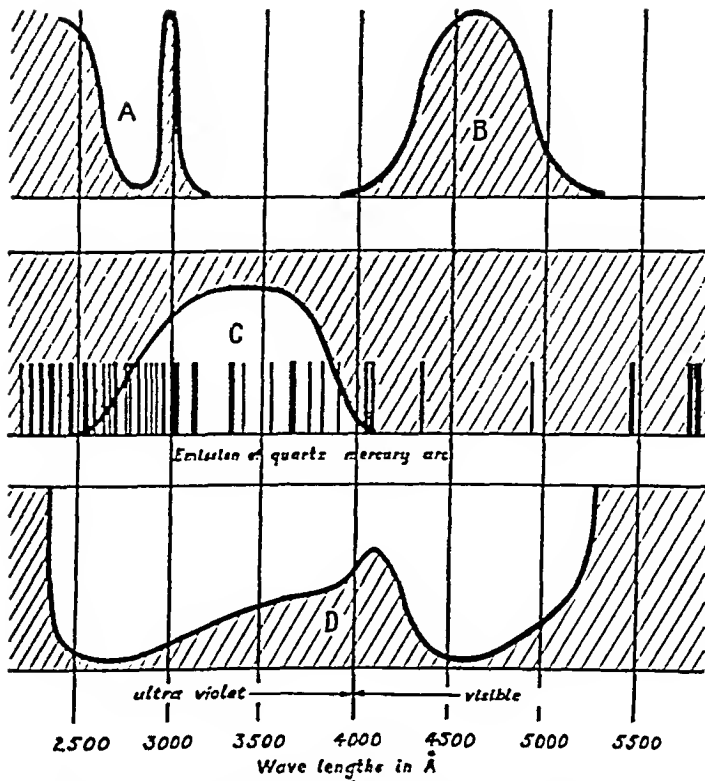


FIG. 2. *A*, spectral region producing normal delayed erythema response (after Luckiesh, Holladay and Taylor (1930)). *B*, spectral region producing abnormal "triple response" in our patient (roughly approximate). *C*, transmission of Corning 986 filter. The approximate spectral position and relative intensity of the lines emitted by the quartz mercury arc are also shown. *D*, absorption spectrum of "hematoporphyrin." (Composite from several porphyrins, after Hausmann and Krumpel (1927).)

the sun's rays. An examination of these data will demonstrate that the active wave lengths lie between 3900 and 5300 Å and that only the region between 4100 and 4900 Å is very active, since only erythema or very slight edema was produced after long exposure to the region between 3900 and 4100 Å, and to that between 4900 and 5300 Å. Thus for rough purposes we may say that the active region lies between 4000 and 5000 Å. Observations using the quartz mercury arc, which are also described in Table I, correspond with those for sunlight. Fifteen minutes of irradiation at 25 cm. distance, with the 4050 Å line virtually removed and all longer wave lengths in the visible region completely eliminated by the use of Corning 986, failed to produce any trace of the immediate "triple response," although it produced a strong delayed erythema followed

by pigmentation. On the other hand, the full radiation from the arc, or only those lines passing through window glass, i.e., above 3200 Å, produced a slight erythema in the same time. Reference to Figure 2C shows the lines of the mercury arc; the general lack of radiation in the spectral region producing the immediate "triple response," i.e., between 4000 and 5000 Å, is evident. By controlling various factors it might be possible to obtain a more exact estimation of the effectiveness of various wave lengths, but as it seemed that little was to be learned from more exact data this study was not carried further.

So far as they go, these observations demonstrate a number of very striking facts. In Figure 2B the active wave length for this response is represented approximately and may be compared with that for the erythema response of normal

the circulation remaining cut off during this period. A corresponding portion of the opposite arm was exposed simultaneously for comparison. After the reactive hyperemia following removal of the cuff had subsided, the two arms were compared; both showed erythema and edema in comparable degree. This was true in a number of experiments. While these results would suggest that  $O_2$  is not necessary for the reaction, they cannot be considered as absolutely eliminating this possibility, since it is impossible to say definitely whether or not a high degree of anoxemia was produced in the skin of the arm, and Blum and Spealman (1934) found that a very low  $O_2$  tension must be established in order to abolish such reactions. Thus the results cannot be accepted as conclusive, although if they were they would indicate that the reaction herein described does not belong to the type of photosensitization produced by hematoporphyrin in the laboratory.

On considering these facts we were not surprised to find that porphyrin could not be demonstrated in the urine of our patient. Duke (1925), Frei (1925) and Vallery-Radot et al. (1926) were likewise unable to demonstrate porphyrin in the urine of their patients.

We must look further for a pigment which will satisfy the requirements, but we have no great hope of success. Bile pigments are reported to be photoactive but their absorption spectrum does not meet the requirements, and moreover our patient showed no indication of jaundice and no excess of bile pigments in the urine. We know of no hem compound which has the required absorption spectra. The "yellow ferment" of Warburg and Christian (1933) has its absorption in the same spectral region which is effective in the production of the immediate "triple response," but any explanation in terms of this compound must be highly speculative. For the present we must be content with evidence proving the existence of a photosensitizing substance without being able to identify it.

*Relationship to other conditions.* As Lewis (1927) has pointed out, many irritating substances may produce the "triple response." Thus the urticarias, while probably all resulting from tissue injury and resultant "H" substance production, may be brought about by quite dissimilar mechanisms. It seems probable that the

present example of photosensitivity has little relationship with the allergies, though the tendency has been to place such cases in this class (see Duke, 1925). The fundamental mechanism would appear to be different, namely, the production of "H" substance following the photoactivation of a particular kind of light-absorbing molecule. The classification of this type of reaction with the "physical allergies," e.g., reactions to 'heat' and cold, by Duke (1925) is perhaps somewhat misleading; actually the problem is a photochemical one and is simplified when considered as such.

The relationship to other types of photosensitivity, e.g., *hydroa*, *eczema solare*, *lupus erythematosus*, etc., is extremely interesting. While it is possible that all such conditions have a common etiological factor, this is by no means a necessary assumption and seems highly improbable. The symptoms are different enough to be given distinctly separate clinical classifications, although the custom seems to differ. Rasch (1926) suggests a relationship between certain of the separately classified conditions, but the identity of a common photochemically active sensitizer has never been definitely established, and in only one condition—that herein described—has the spectral region of absorption of such a sensitizer, i.e., the effective wave lengths, been clearly delimited.

Duke (1923) has described a case in all ways comparable with ours, in which the response was likewise elicited by violet and blue light. The case described by Frei (1925) and the two by Vallery-Radot et al. (1926, 1928) were also definitely sensitive to violet and blue light, and the latter investigators delimited the spectral region between 4000 and 5500 Å (1926), which agrees with ours within the accuracy of the methods. One of Wucherpfennig's (1928) cases (designated HW.) probably belongs to this group. Although the active wave lengths given by this author do not agree exactly with ours, there seem to be objections to his method of measurement which may account for the apparent difference. The cases described by Ward (1905) and by Cummings (1926) were also sensitive to wave lengths greater than 3200 Å,<sup>4</sup> but the spectral region was not delimited further than by the ob-

<sup>4</sup> Through window glass.

servation that the patients were sensitive through window glass. Their cases probably represent, however, the same condition as that described herein.

The case which Beinlauer (1925) studied and described as *urticaria solaris* manifested an immediate "triple response" and was in this respect similar to the cases mentioned above. However, in that case the response was elicited by an altogether different part of the spectrum, i.e., by wave lengths shorter than 3200 Å.<sup>5</sup> Therefore, it must have been etiologically different. Two of Wucherpfennig's (1928) cases (designated Pa. and Mi.) may or may not belong to the same category as Beinlauer's but are certainly not the same as ours, Duke's or Vallery-Radot's.

The case of Weiss (1932) was apparently distinct from ours in that the urticarial reaction was delayed about four and one-half hours, and that it was produced by quartz mercury arc radiation, which was barely effective in our case.

In the very interesting series of Barber, Howitt and Knott (1926) all the patients appear to have shown some degree of eczema, which was absent in our case and in those to which reference has just been made. It would seem proper, therefore, to classify their cases as *eczema solare*. Unfortunately, the investigations by these authors of the spectral region of the radiation evoking the response in the skin were not exact enough for us to compare this feature of their cases with ours. It would appear from their data that some of their patients were sensitive to visible radiations, probably violet and blue, and some to ultraviolet, but one cannot be sure. The case of *eczema solare* described by Goeckerman, Osterberg and Sheard (1929) seems to have been sensitive to wave lengths in the region of 3000 Å, which is the region producing the erythemic response of normal skin.

As stated above, the wave lengths producing *hydroa* have never been definitely determined. Gottron and Ellinger (1931) have recently reviewed the material on this subject and have added another case. The results are in general somewhat confusing; it would seem that while

some patients with *hydroa* show a greater sensitivity to ultraviolet radiations, others may show an increased tolerance to such radiations. Unfortunately, few of the investigators of this condition have used sunlight in their tests; yet this is the radiation to which the patients are most frequently exposed and it is the most powerful source of visible light readily at hand.

From this brief resumé it would appear that no common etiological factor for photosensitivity in man has been demonstrated, and that there almost certainly exist more than one. Since the etiological factor in photosensitization must be the photoactive compound which absorbs the light, it would seem highly important to attempt to characterize this factor in terms of the wave lengths of light which produce the clinical effects. The determination of this spectral region is as important in the diagnosis and classification of these diseases as is the isolation of the microorganism associated with a bacterial disease.

Since these cases are rare, it is hoped that they will be carefully studied when discovered. The writers would greatly appreciate receiving reports of any such cases and would gladly cooperate in giving advice or assistance where possible.

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<sup>5</sup> Quartz mercury arc radiation was effective through "nickel oxide glass" (probably the same type as Corning 986) but not through window glass.

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# THE EFFECT OF RENAL DENERVATION ON PATIENTS SUFFERING FROM NEPHRITIS<sup>1</sup>

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Jaboulay (1) appears to have been the first to perform the operation of periarterial neurectomy in man. The procedure, however, did not achieve popularity until after the appearance of a series of papers by Leriche and his associates (2, 3). The effect on patients suffering from peripheral vascular disease has been especially extensively investigated. Denervation of the kidneys more recently has been performed on patients suffering from nephralgia and hydronephrosis, success being achieved in the relief of pain.

## ANATOMICAL

The renal nerve supply is derived mainly from the celiac ganglia with small branches from the plexuses around the adrenal gland and the aorta. Usually one branch comes from the splanchnic nerves direct. Through the celiac ganglion the renal nerves are brought into connection with the splanchnic nerves and with the vagus. The vagus nerve also sometimes sends a direct branch to the kidneys. A few branches are received from the superior mesenteric ganglion.

It has long been recognized that the splanchnic nerves form the chief vasomotor supply of the kidneys. Bradford (4) found that stimulation of the anterior roots of the cord from the sixth thoracic to the second lumbar segment caused the kidneys to constrict. Of these roots the tenth to twelfth dorsal were most effective. Vasodilator fibers were also present but were weak in comparison with the constrictors.

Langley and Anderson (5), Jost (6), and Hirt (7) have clearly demonstrated fibers coursing from the third, fourth, and fifth lumbar ganglia to join the renal plexus. Most of the nerves are non-medullated, but some in the plexus are myelinated (Renner (8)). Ganglion cells which are

numerous within the plexus are not found in the renal parenchyma.

Smirnow (9) observed that the nerves follow the blood vessels into the renal tissue even to the smaller capillaries. Motor nerve endings were seen in the smooth muscle of the vessels, as well as small nerve fibers in the capillary tuft of the glomeruli, the glomerular membrane and in the tubules. Sensory nerve endings occurred in the smooth muscle of the pelvis and in the connective tissue of the adventitia and media of most renal vessels and the capsules. Secretory nerves to the kidneys have never been convincingly demonstrated (Cushny (10)).

## PHYSIOLOGICAL

Section of the splanchnic nerves in animals was shown by Claude Bernard (11) to be followed by increased excretion of urine more dilute than normal. This observation has been confirmed many times (Eckhard (12), Klecki (13), Grek (14), Rohde and Ellinger (15), Asher and Pearce (16), Jungmann and Meyer (17)). It also increases markedly the elimination of chlorides and carbonates and to a less extent that of urea, phosphates and sulfates. Elimination of creatinine, ammonia and phenolsulfonphthalein is unchanged according to Marshall and Crane (18). Section of the splanchnic nerves in frogs causes more glomeruli to fill with blood than in the control kidney (Bieter (19)). Should polyuria, increased blood flow and increased excretion of salt and urea, occur following denervation of the kidneys in patients, it would be of benefit.

Burton-Opitz and Lucas (20) showed that the vasoconstriction following stimulation of the splanchnic nerves or renal plexus occurs only on the side stimulated. Complete section of the renal nerves in animals was probably first performed by Carrel and Guthrie (21, 22). They were able to transplant both kidneys from one

<sup>1</sup> Delivered at the International Medical Assembly, Interstate Postgraduate Medical Association of North America, Philadelphia, November 8, 1934.

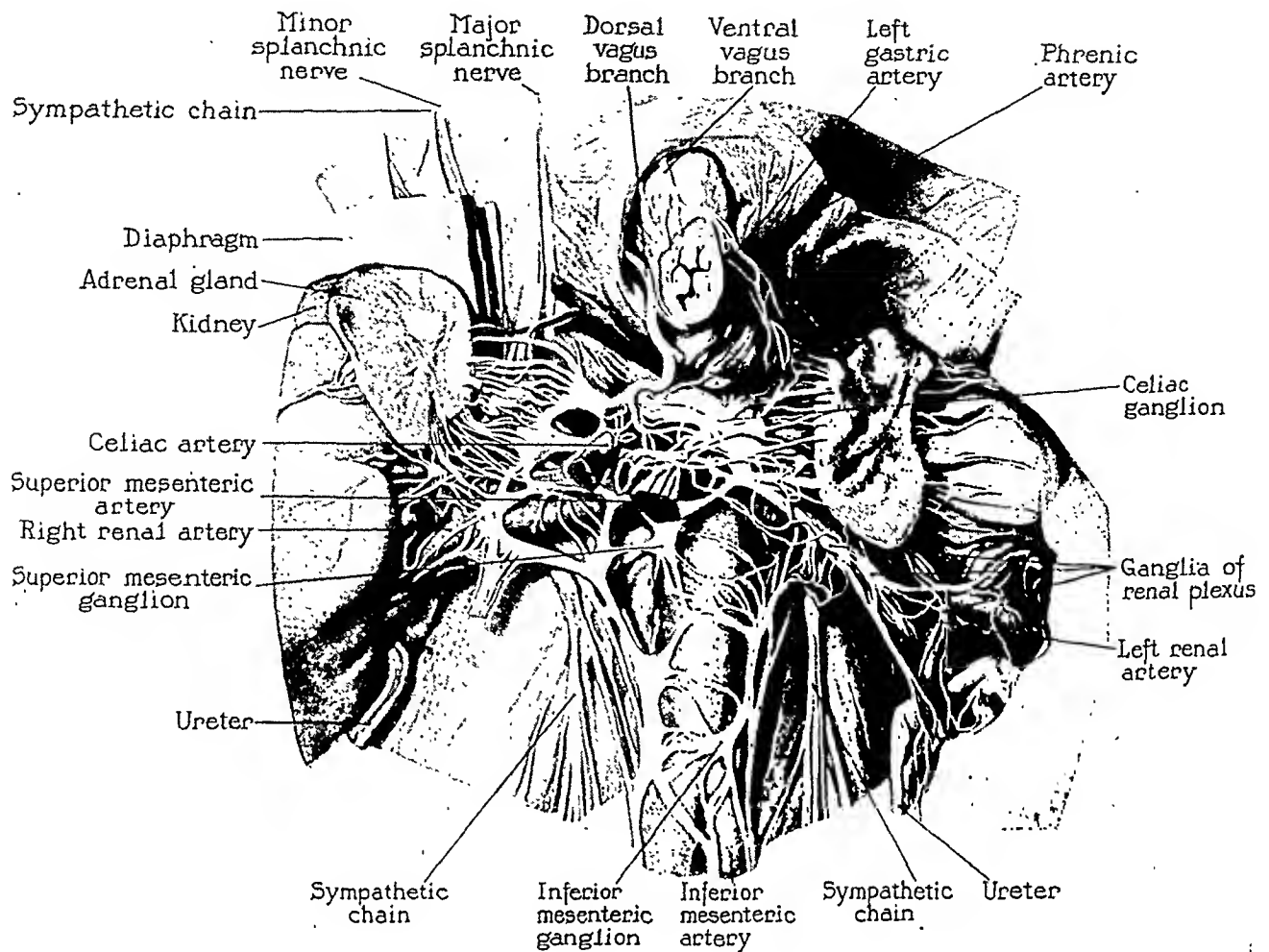


FIG. 1. RENAL NERVES IN MAN.

From A. Hirt, *Ztschr. f. Anat. u. Entwicklung Gesch.*, 1924, 73, 621.

dog to another after having removed both kidneys from the latter. Except for some protein, the urine excreted was normal. This work was confirmed by Dederer (23, 24), Ibuka (25), and Holloway (26), who further showed that homeo-transplants function adequately for some time.

Lobenhoffer (27) found that transplanted kidneys remain histologically normal at least for a few weeks, nor was there any difference in transplanted and normal kidneys in their ability to excrete water and sodium chloride. Diuresis induced by hydremia showed that vascular efficiency was maintained.

Animals in which total hypophysectomy followed by decerebration had been performed did not exhibit increased flow of urine or any marked change in the output of chloride (Fee (28)). Denervation of the kidneys, however, resulted in the production of large amounts of hypotonic

urine. Administration of pituitrin reduced the increased output to normal. Bayliss and Fee (29) found that perfusing innervated kidneys with heart-lung preparations, unlike isolated perfused kidneys, did not excrete large amounts of hypotonic urine until denervation was effected.

Renal denervation, just as splanchnic section in normal dogs, results in diuresis which may persist for months (Rohde and Ellinger (15), Stierlin and Verriotis (30), Caldwell, Marx, and Rowntree (31)), or not more than two weeks (Quinby (32)). The total amount of water, chlorides and urea eliminated was greater on the denervated side, and with little change in the amount of creatinine and phenolsulfonphthalein (Marshall and Kolls (33), Milliken and Karr (34), Kusakari (35)).

Hecht's experiment (36), in which oil was injected into the aorta after tying the vessel distal

to the renal arteries, showed that more oil entered the vessels of the denervated than the normal kidney. Milles, Müller and Petersen (37) also found by x-ray examination of injected kidneys that denervation caused chronic vasodilatation.

Quinby (32) tested the response of denervated kidneys to intravenous injection of hypertonic solutions of sodium chloride, urea and caffeine, and found that the reactions were practically identical with those of normal kidneys.

Rhoads, Van Slyke, Hiller, and Alving (38) have shown that infiltration with novocaine or section of the renal nerves in dogs was without consistent effect on either the excretory efficiency of the kidney, as measured by the urea clearance, or on the renal blood flow. There appeared, nevertheless, to be a possibility that the renal blood flow, and with it the function, might be increased in nephritic patients by denervation.

Müller and Petersen (39) observed that denervation prevented infection of kidneys by colon bacilli infused intravenously. X-ray photographs taken after injection of an opaque medium showed that dilatation of the larger arteries had occurred on the denervated side. Chilling animals caused constriction of the renal vessels in the innervated but not in the denervated kidneys. (Milles, Müller, and Petersen (40)). If the chilling was repeated daily, morbid changes occurred in the vascular bed of normal kidneys, conversely denervated kidneys remained unaltered. Clinical observation has implicated chilling of the skin as an important cause in the initiation of nephritis and in the appearance of exacerbations. Physiological evidence indicates that the caliber of the vessels of the kidneys follows closely that of the skin vessels (Cohnheim and Roy (41), Wertheimer (42)). Denervation in patients might dissociate these functions as it does in animals.

Hecht (43) found that suspensions of clumped, attenuated *Staphylococcus aureus* injected into the veins of rabbits caused more micotic abscesses to develop in normal than in denervated kidneys. If denervated kidneys are less susceptible to infection than are normal ones, denervation might be of therapeutic value in some conditions.

Normal kidneys excrete intravenously injected *Bacillus prodigiosus* in a wave-like fashion. Following denervation the number excreted was

greatly reduced and the excretion became continuous (Milles and Nedzel (44)). It is possible that intermittency of excretion was due to periodic activity of the glomeruli (Richards and Schmidt (45)).

Kidneys of dogs following injection of colon bacilli, were not apparently injured for a period of about 30 minutes but, with the onset of chills, albumin, red blood cells and bacteria appeared in the urine (Müller, Petersen and Rieder (46)). If one kidney had been denervated, it continued to excrete normal urine after the injection of bacteria in spite of chills. Moriconi (47) was unable to prevent the appearance of suppurative nephritis in dogs by decapsulation and denervation.

It has been a favored theory that arterial hypertension in nephritis is due to nervous reflexes originating in the kidneys. Experiments performed on dogs by Page (48), in which renal hypertension had been produced by constricting the renal artery (Goldblatt, Lynch, Hanzal and Summerville (49)), did not support this view. He showed that hypertension resulted even when the nerve supply to the kidneys was severed. It was considered important to ascertain in patients whether the renal nerves were concerned in the genesis of nephritic hypertension.

#### PREVIOUS CLINICAL DATA

Temporary partial denervation of the kidneys has been attempted by means of paravertebral injections of local anesthetics. Wiedhopf (50) found that blocking the 11th and 12th dorsal and the first lumbar segments in patients produced an increase in urine and sodium chloride excretion, but Lurz and Röhrich (51) were unable to confirm this. Three cases of reflex anuria were thought by Haslinger (52) to be benefited by paravertebral injections of novocain. Splanchnic anesthesia also has its advocates in the treatment of reflex anuria (Neuwirt (53), Havlicek (54)).

Renal denervation in man was probably first performed by Papin (55) and by Legueu and Flandrin (56) for relief of pain in the kidneys. Since that time a number of urologists have performed the operation for nephralgia and painful conditions arising from contractions of the renal pelvis (Harris and Harris (57), Herbst (58), Hess (59), Stone (60)). Milliken and Karr



(34) and Hess (59) from theoretical considerations gave the following indications for renal denervation:

1. Nephralgia, with or without accompanying non-obstructive hydronephrosis.
2. Unilateral hematuria without evidence of renal pathology.
3. As an adjunct to the removal of stones in the pelvis, with the hope that denervation may prevent subsequent recurrence.
4. Anuria that lasts longer than a few hours.
5. Early tuberculosis.

Rieder (61) has reported the results of unilateral denervation in the case of a patient suffering from what appeared to be early malignant sclerosis. The blood pressure was 240/110 mm. Hg, the nonprotein nitrogen 52 mgm., and the urine contained red blood cells and albumin. There were no eye-ground changes. Nine months after operation the blood pressure was 140/90 mm. Hg, and the nonprotein nitrogen in the blood was normal. The urine excreted from the denervated kidney was less concentrated than that from the other kidney. Subjectively the patient was much improved.

If, as Volhard (62) believes, nephritis is caused by spasm of the vessels within the kidneys, provided the spasm is maintained by the nerves to the vessels, denervation should relieve it and increase the blood flow. General experience with the operation of denervation indicates that interruption of the nerve supply to vessels which are spastic restores normal tone, but that denervation of vessels with normal tone has little effect.

Page and Heuer (63) have shown that denervation of the kidneys in a patient with essential hypertension did not change the renal efficiency as measured by the urea clearance nor did it significantly reduce the ability to concentrate urine. Measurements performed before the operation indicated that renal function was normal.

#### *Selection of patients for operation*

Our intention was to select patients in whom nephritis had become chronic and progressed so far that, on the basis of previous observations (Van Slyke, Stillman, *et al.* (64)), further progress to the terminal stage appeared certain unless corrected by therapeutic means. In order to

avoid too great hazard in the operation, however, cases were chosen in which the urea clearance was still well above the 20 per cent level, which marks entrance into the terminal stage. The onset of the nephritis had occurred 16 months before operation in the earliest case and 26 months in the latest. Evidence of the clinical condition is given by the graphic charts.

#### METHODS OF STUDY

The patients were studied in the hospital several months before operation was performed. Reliance for studies of renal efficiency was placed on the urea clearance test of Møller, McIntosh, and Van Slyke (65), not only because it is the most delicate test, but because Van Slyke, Rhoads, Hiller and Alving (66) have shown that in dogs it parallels the renal blood flow. The ability of the kidneys to concentrate urine was ascertained by the technique of Addis and Shevky (67), in which one measures the specific gravity of a 12-hour specimen of urine passed during the latter half of a 24-hour period, during which the patient received no fluids. A Westphal balance, modified by Alving and Van Slyke (68), was employed for the determination. Correction was made, as described by Lashmet and Newburgh (69), for the gravity contributed by the protein present in the urine. The figures thus represent nonprotein specific gravity of maximum concentration. Urinary protein was estimated by the Shevky-Stafford method (70). Red blood cells and casts were counted according to the technique of Addis (71). The hemoglobin of the blood was recorded in terms of volumes per cent of oxygen capacity. Plasma proteins were ascertained by the method of Howe (72). Plasma lipids were measured by the method of Kirk, Page and Van Slyke (73). Blood pressure measurements were made at about 9:30 a.m. with the patient in bed. On the charts these measurements for each week are averaged for convenience in representing them. Changes in the eyegrounds are recorded as follows: (1) constriction of the arterioles, (2) arteriosclerosis, (3) exudate, (4) hemorrhages, (5) papilledema. The estimated time elapsing between onset of the disease and admission to the hospital is recorded in the lowest line on the charts, as the first number following the word "months." Diets were for the most part relatively high in protein and constant in caloric intake. They were supplied by a diet kitchen.<sup>1</sup>

#### *Operative procedure*

The technique of bilateral renal denervation as we have performed it may briefly be described as follows: Under general anesthesia the usual kidney incision is made upon the right side, the perirenal fat separated, and the kidney exposed. With great care, in order to avoid bleeding,

<sup>1</sup>We wish to thank Miss G. Drew for her skillful management of the diets.

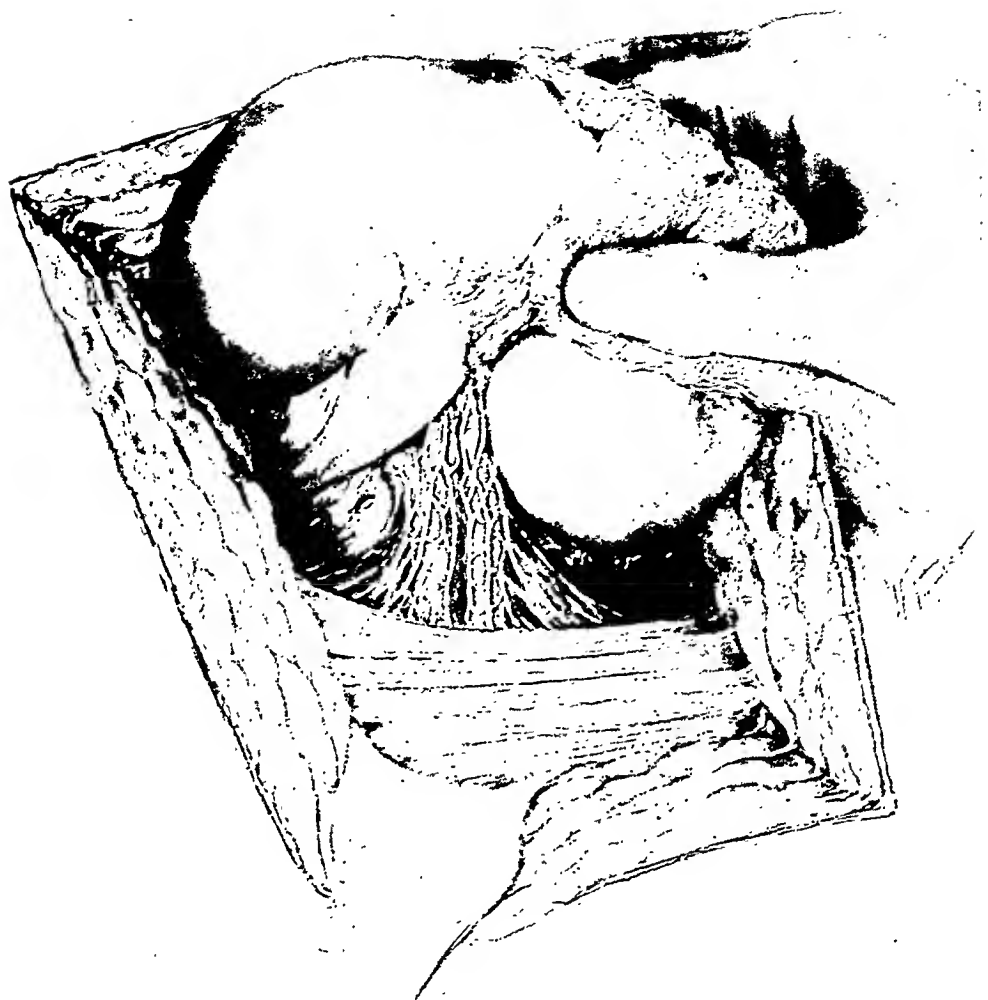


FIG. 2. OPERATIVE EXPOSURE OF THE KIDNEY SHOWING THE NERVES OF THE RENAL PEDICLE.

the fat is stripped away from the kidney, which is gradually completely freed and drawn well outside of the body. The ureter, renal artery, and vein having been identified, the renal vessels are stripped of all fat downward to their point of origin. If the freeing of the vessels is meticulously performed, the operator then will plainly see the sympathetic nerves coursing from their origin to the kidney. They appear as a fan-shaped, triangular mass upon both the dorsal and ventral aspects of the renal vessels, the base of the triangle being directed toward the aorta and vena cava, the apex converging upon the renal vessels. Upon reaching the renal vessels, the nerves apply themselves more closely to them and surround them in a more or less well defined basket weave plexus.

The nerves having been identified their excision is begun. Beginning upon the ventral aspect of the vessels exposed by drawing the kidney backward, the fan-shaped mass of nerves is carefully freed and divided near the vena cava. The mass is grasped, drawn upward and outward, and the nerves stripped from the artery and vein up to their entrance into the kidney. Having completed the removal of the nerves from the ventral aspect of the vessels, the kidney is drawn forward and a similar resection of the nerves upon the dorsal aspect of the vessels is performed. These two procedures remove practically all the nerves to the kidney; but there may remain a few which are applied to the opposing surfaces of the artery and vein. These vessels are therefore separated, and a search made for any remaining nerves. If found they

are resected. At the completion of the operation the artery and vein should appear as naked, isolated structures completely stripped of all nerves. The kidney is then replaced in its fatty bed, and the wound closed. Two weeks to a month or more later a similar operation is carried out upon the opposite side.

The operation has been well borne both during and after the procedure. Neither the renal artery or vein has been injured in any patient thus far subjected to operation, nor has there been a single incident to mar the postoperative convalescence. Experience has shown that adequate exposure is of the greatest importance in the performance of the operation, and we have, therefore, when operating upon the left side, unhesitatingly divided the twelfth or the twelfth and eleventh ribs in order to secure it.

#### PROTOCOLS

*Case 1.* (See Figure 3.) Hospital Number 8333, (E.McG.), female, age 57 years. The onset of the disease was insidious in character seven months before admission to the hospital. She complained of severe headaches, nausea and scanty passage of urine. Dr. E. Mayne found hematuria, albuminuria, edema, and the blood pressure elevated to 210 mm. of Hg. Rest and a low protein, low salt diet did not improve her condition, and as she appeared to be rapidly getting worse she was referred to the hospital by Dr. Mayne. Edema had been especially severe and troublesome.

The physical examination disclosed that the eyegrounds were normal except for a small fresh hemorrhage in the right retina. The heart was very slightly enlarged. The radial vessels were thickened and slightly tortuous. Pitting edema was observed up to the knees. Blood pressure was 198/104 mm. of Hg. Inhalation of amyl nitrite caused it to fall 58 mm. of Hg systolic and 40 mm. diastolic. The electrocardiogram showed all the T waves positive, conduction time 0.16 seconds, moderate left ventricular preponderance.

Twenty-three months after the onset of the disease it was clear that the urea clearance was stabilized at about 50 per cent of normal and that maximum concentrating ability was 1.020. The hemoglobin rose to normal and hematuria disappeared. Total plasma protein also approached normal, but the albumin fraction remained at 3 per cent. Excretion of protein in the urine fluctuated little from 5 grams in 24 hours. The blood pressure varied from 160 to 200 mm. of Hg systolic and 80 to 110 mm. diastolic.

Right renal denervation was performed March 3, 1934, during the 24th month of the disease. The kidney was bound down by many dense adhesions but was found to be of normal size. It was moderately scarred, and the renal arteries were smaller than normal, were beaded, and showed definite sclerosis. The masses of nerves removed showed on section that the majority were non-myelinated, but a few myelinated nerves were present. The postoperative course was uneventful, and at no time

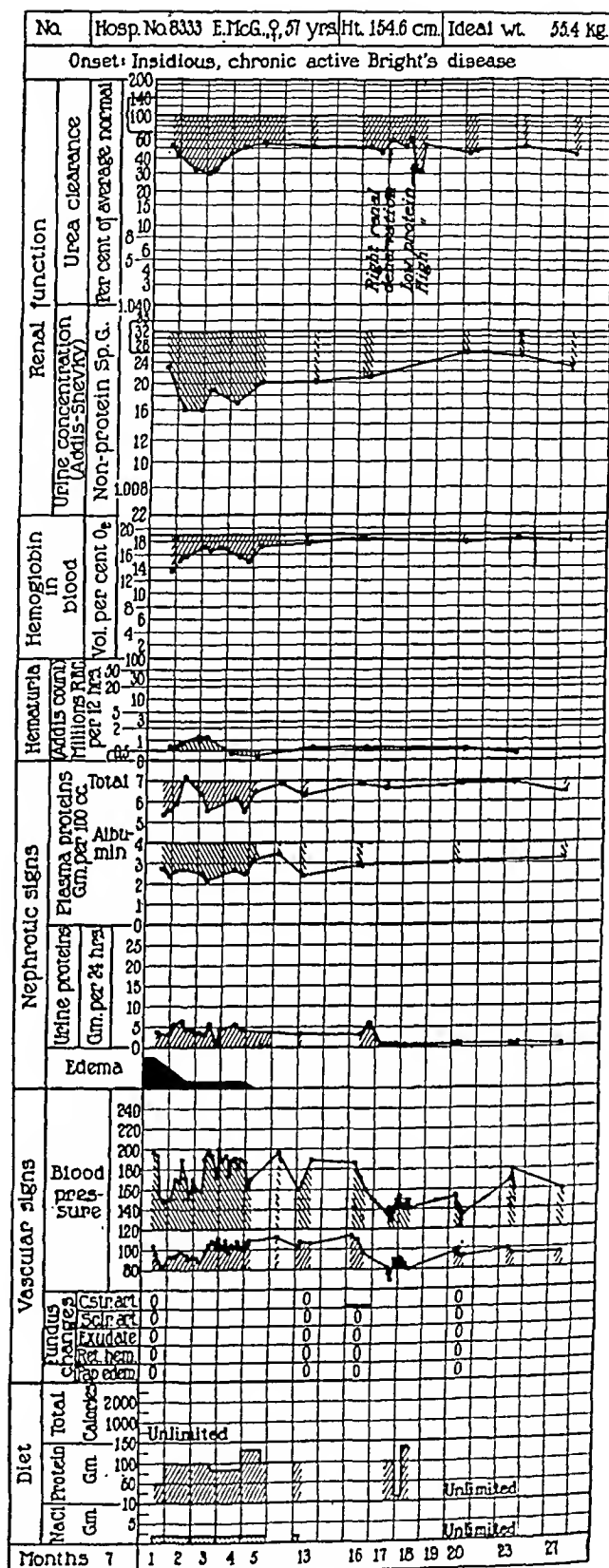


FIG. 3. UNILATERAL DENERVATION IN PATIENT WITH CHRONIC ACTIVE NEPHRITIS.

did oliguria develop. The urea clearance test performed 18 days after operation showed that it had improved slightly. About three months later cystoscopy was done by Dr. O. Lowsley, and the urea clearance was per-

formed on specimens from the catheterized ureters. The results are as follows:

	Time	Cor- rected urine volume per minute	Urea N	Blood urea N	Clear- ance in per cent of nor- mal
	minutes		mgm.	mgm.	
Denervated kidney...	30	.62	580.0	23.49	36.2
Control kidney.....	30	.59	528.0		31.9

Six months after operation the urea clearance had not changed from the preoperative level. Ability to concentrate urine rose from 1.020 maximum nonprotein specific gravity to 1.027. The protein excretion in the urine fell sharply to less than one gram in 24 hours. The blood pressure fell and remained at almost 150/90 mm. of Hg. but has shown a definite tendency to return to the preoperative level. Subjectively the patient feels much improved and has returned to her household and social activities. We have not urged the denervation of the left kidney.

*Case 2.* (See Figure 4.) Hospital Number 8634. (E.G.) female, aged 22 years. The onset of the disease was acute three months before admission to the hospital. She suffered from a severe sore throat and two weeks after its onset woke up one morning to find edema of her face, hands and feet. Two days later ascites developed. Hematuria and albuminuria were found, and the blood pressure was 150/90 mm. of Hg. The patient began to vomit and suffered from severe headaches; consequently she was referred to this hospital by Dr. H. Greisman.

Physical examination disclosed marked edema of the legs and a small amount of fluid in the abdomen. In both eyegrounds there were numerous old and fresh hemorrhages. Anemia was very marked, and the blood pressure was 158/98 mm. of Hg. Gastric analysis showed that there was no free HCl in the fasting specimen and none 40 minutes after alcohol, but 33 after histamine. Total acid in the fasting specimen was 3.1, 11.4 after alcohol and 47.0 after histamine. The pH of the serum was 7.24,  $\text{CO}_2$  17.29 millimols per liter, chlorides 109 milli-equivalents, and total base 148.2 milli-equivalents per liter. Blood lipids were as follows: total fat 1896 mgm., total cholesterol 718 mgm., free cholesterol 194 mgm., ester cholesterol 524 mgm., lipid amino-nitrogen 12.5 mgm., lipid phosphorus 19.8 mgm., total lipid nitrogen 35.5 mgm. per 100 cc. of plasma.

One month after admission paravertebral anesthesia was administered by Dr. H. Wertheim at Dr. A. Alving's suggestion. Novocain was injected on both sides of the vertebrae from the ninth thoracic to the first lumbar segments. Two hours prior to the injection the clearance was 13.9 per cent of normal. Two hours after the anesthesia it was 15.2 per cent and 14.1 during the next two hours. Evidently paravertebral novocain anesthesia had

no effect on the renal blood flow as measured by the urea clearance test.

During the next seven months, as shown by Figure 2, urea clearance and hemoglobin rose steadily. Thereafter, however, no further improvement occurred, and it became evident that the case had become chronic. The most concentrated urine the patient could excrete had a specific gravity of 1.012, and there was only a slight increase associated with the rise in urea clearance. Other data are evident from the chart.

Right renal denervation was performed on May 23, 1934, in the sixteenth month after the onset of the disease. The kidney was rather large and pale, measuring 12 cm. in length, 6.5 cm. in width at the pelvis and 4.5 cm. in thickness. It was moderately scarred, but the main renal vessels were quite normal in appearance.

Twenty-one days after operation the patient was cystoscoped, and the urea clearance test was performed on the urine collected from the ureteral catheters.

	Time	Cor- rected urine volume per minute	Urea N	Blood urea N	Clear- ance in per cent of nor- mal
	minutes		mgm.	mgm.	
Denervated kidney...	30	0.77	417.0	32.8	21.1
Control kidney.....	30	0.58	438.0		18.8

Studies made during the next four months showed that unilateral denervation caused no significant change in the urea clearance, ability to concentrate urine or the hemoglobin. Hematuria markedly diminished, and plasma proteins rose very slightly. Excretion of protein in the urine diminished about 60 per cent. Changes in blood pressure level were not considered significant. The patient was edema-free even though salt was allowed in the diet. Determination of plasma lipids showed that a marked fall had occurred. Lipids in 100 cc. of plasma were: total fat 1220 mgm., total cholesterol 535 mgm., free cholesterol 238 mgm., ester cholesterol 297 mgm., lipid amino-nitrogen 4.6 mgm., lipid phosphorus 14.2 mgm., and total lipid nitrogen 17.3 mgm.

The left renal denervation was performed on October 2, 1934, about four months after the initial operation. The kidney was smaller than on the right, measuring 10.3 x 5 x 4 cm. There appeared to be no evident change in the clinical or laboratory findings over those observed following the first operation.

*Case 3.* (See Figure 5.) Hospital Number 8860. (J. S.), female, age 22 years. Two months before admission the patient noticed that her feet and ankles were swollen. One month before this she became pregnant. Four months later she was admitted to the Presbyterian hospital because of ascites. Excretion of urine had diminished, and she complained of palpitation, substernal oppression and transitory loss of vision. The Wasserman test was negative. Much protein and a few red blood cells were found in the urine. It was necessary to

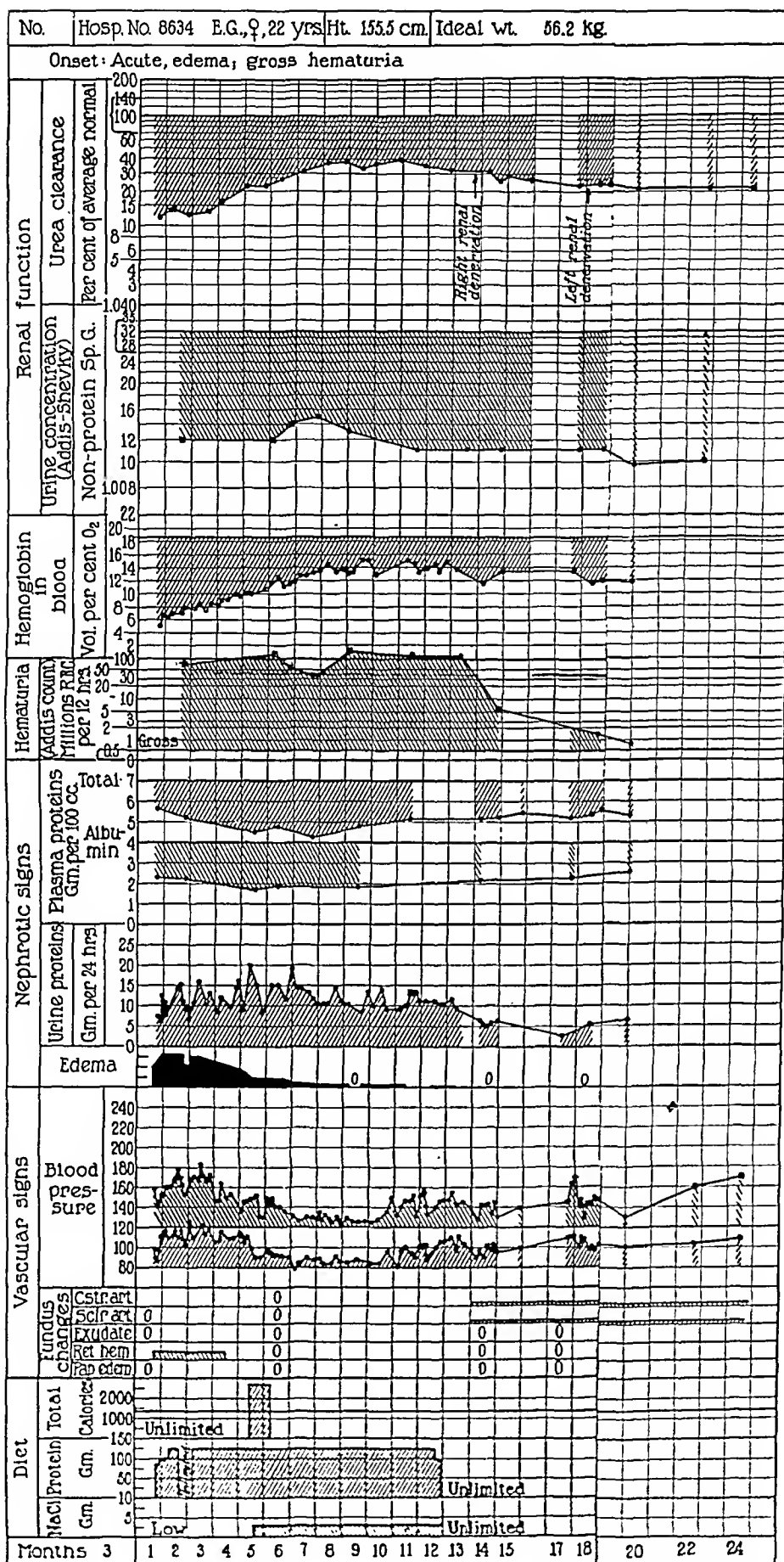


FIG. 4. BILATERAL DENERVATION IN PATIENT WITH CHRONIC ACTIVE NEPHRITIS.

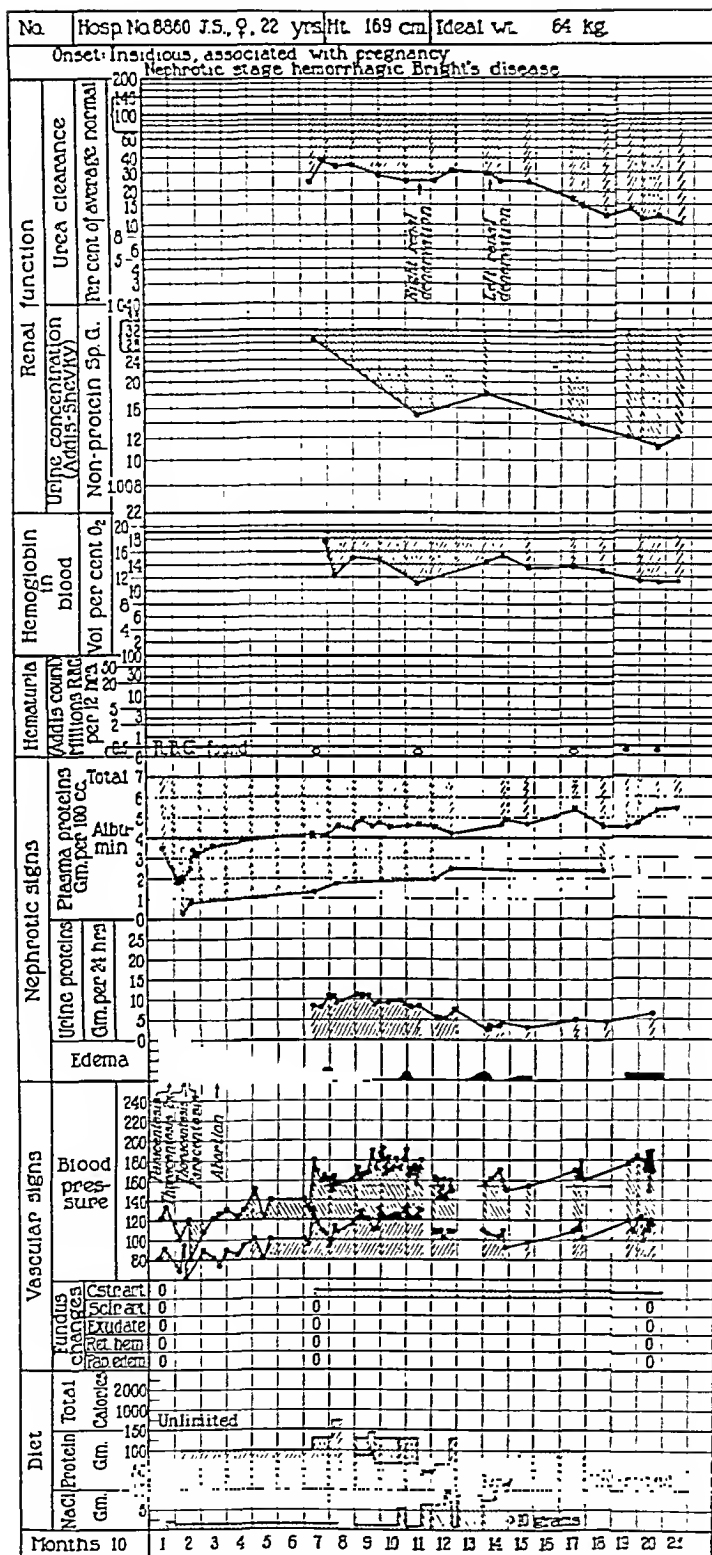


FIG. 5. BILATERAL DENERVATION IN PATIENT WITH CHRONIC ACTIVE NEPHRITIS IN THE NEPHROTIC STAGE.

perform thoracentesis and paracentesis to remove some of the fluid. Intravenous injections of gum acacia were given without any beneficial effect being observed. Her basal metabolism was found to be  $-18$  per cent. Colitis developed with diarrhea and bloody stools. Because of the patient's serious condition an abortion was performed. About one month later phlebitis of the left arm appeared, which healed with conservative treatment. A week later the patient was sterilized by resection of the tubes. The right kidney was seen to be much enlarged, while the left was about normal in size. Three months later she was discharged from the hospital but returned in two days because severe edema immediately developed. Her basal metabolism was then  $-24$  per cent. We are indebted to Dr. A. Harris and Dr. W. Anderton for this information.

Physical examination disclosed marked anemia, edema and malnutrition. The eyegrounds were normal except for beginning arteriosclerosis. Blood pressure was elevated to 180/130 mm. of Hg.

The electrocardiogram showed the T waves to be negative in Leads I and II. Conduction time was 0.16,  $R_3$  and  $S_3$  were split, and left ventricular preponderance was present. Renal efficiency was markedly impaired. The first examination indicated that she had retained power to concentrate urine, but four months later the maximum nonprotein specific gravity of the urine was 1.015. Marked anemia was found, and the plasma proteins were reduced to well below the level at which nephrotic edema appears. Excretion of protein in the urine averaged about 10 grams in 24 hours.

The patient was fed a high protein, low salt diet. A small rise in plasma protein occurred, and much of the edema slowly subsided. The renal efficiency, however, appeared to be gradually diminishing, and during four months marked loss of ability to concentrate urine occurred. Blood pressure also increased.

Right renal denervation was performed on April 18, 1934, in the 21st month after onset of the disease. The kidney was congested, hyperemic and large, measuring  $12.5 \times 7 \times 4.5$  cm. in length, breadth and thickness. The main arteries appeared constricted.

The urea clearance and ability to concentrate urine rose slightly after the operation. Hemoglobin and plasma proteins also were increased, and excretion of protein in the urine diminished by about 50 per cent. No significant change in blood pressure occurred.

On July 6, 1934, about seven weeks after the first operation, denervation of the left kidney was performed. Again this kidney was found to be large, lobulated and perhaps somewhat pale. It measured  $11.5 \times 6 \times 5$  cm. in its various diameters. Directly following this operation no significant change was observed in the clearance, hemoglobin, blood pressure or urinary protein excretion. The patient was discharged from the hospital and lived in surroundings very adverse to her health. She drank rather heavily and was unable to take any care with her diet. She suffered from a series of colds. Examinations made at intervals showed that the renal function was gradually being lost. Plasma proteins have re-

mained about 5 per cent, and the excretion of protein in the urine has continued at a reduced level.

Case 4 (See Figure 6). Hospital Number 8740 (G.

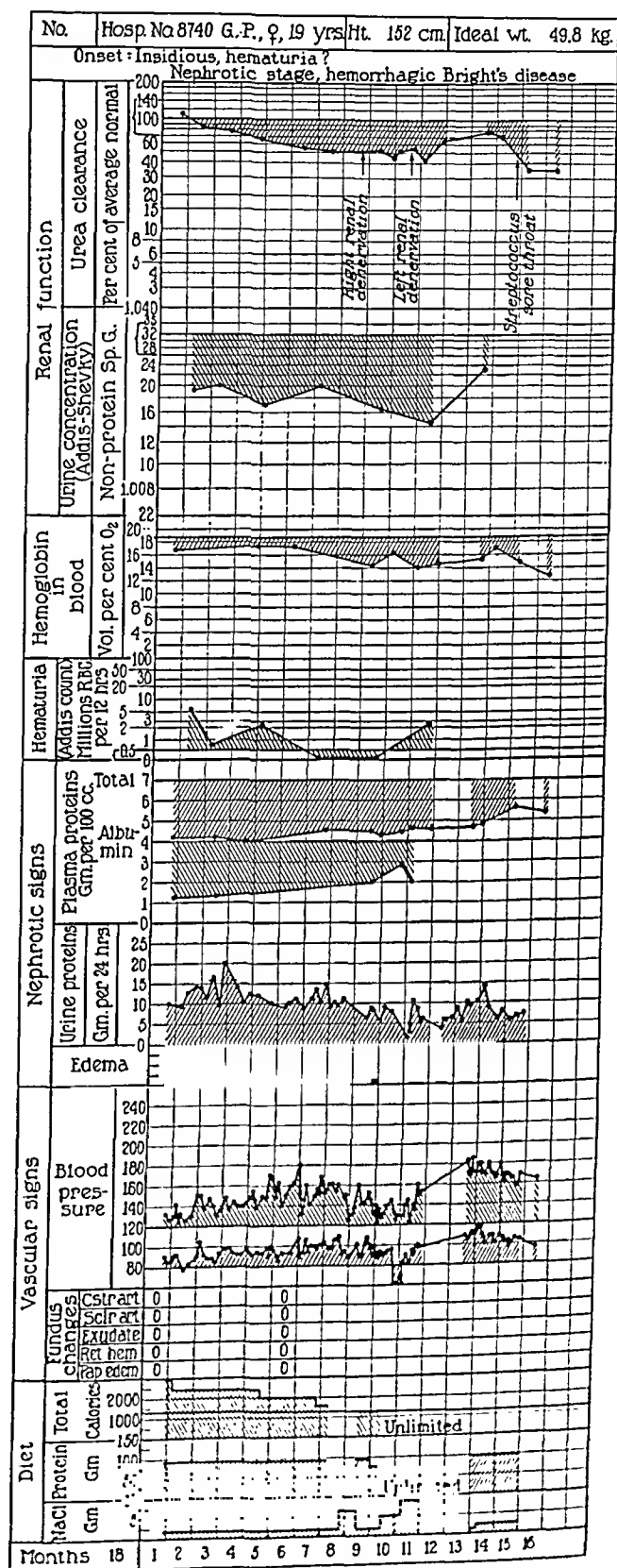


FIG. 6. BILATERAL DENERVATION IN PATIENT WITH CHRONIC ACTIVE NEPHRITIS IN THE NEPHROTIC STAGE.



P.), female, age 19 years. Eighteen months before admission she noticed gradually increasing swelling of her ankles. Albumin and red blood cells were found in her urine. After a short rest in bed she resumed her work. Edema reappeared and during the next eighteen months was almost constantly present.

Examination showed that she was very pale. Her face and legs were swollen with edema. Fluid was present in her chest and abdomen. Eyegrounds were normal. The blood pressure was 130/90 mm. of Hg.

The urea clearance was 103 per cent of normal on admission, but the maximum nonprotein specific gravity of the urine was 1.019. Hemoglobin was moderately reduced, and about 5 million red blood cells were being excreted in 12 hours in the urine. Excretion of protein ranged from 10 to 20 grams in 24 hours. Plasma proteins were severely depleted to 4.09 per cent total protein, albumin 1.19, globulin 2.90, and A/G ratio 0.41. Serum chloride was 103.7 milli-equivalents per liter, and total base of serum 149.6 milli-equivalents per liter. Lipids in 100 cc. of plasma were as follows: total fat 2121 mgm., total cholesterol 589 mgm., free cholesterol 442 mgm., ester cholesterol 147 mgm., lipid amino-nitrogen 6.64 mgm., total lipid nitrogen 17.75 mgm., lipid phosphorus 11.65 mgm. Liver function was normal as measured by the rate of deamination and urea formation by Kirk (74). The Wassermann test was negative.

Renal efficiency fell progressively even though she was confined to bed. Hematuria and proteinuria continued, and the blood pressure rose slowly, reaching a maximum of 180 mm. systolic and 110 mm. diastolic.

On April 26, 1934, twenty-six months after onset of the disease, denervation of the right kidney was performed. The kidney was large and pale and measured 12.5 x 6.5 x 5 cm. in its various diameters.

Shortly before operation the urea clearance was 52.3 per cent of normal, and eighteen days after, was 54.0 per cent of normal. Edema which constantly had been present disappeared promptly following operation, although unrestricted amounts of salt were allowed instead of the previous low salt diet. No significant change occurred in her ability to concentrate urine. A slight fall in hemoglobin appeared, presumably due to the operation. No significant rise in plasma proteins occurred, although some diminution in proteinuria was observed.

Blood pressure fell somewhat. Clearance tests performed on the urine from the catheterized ureters showed that there was no significant difference in the clearance of the denervated as compared with the other kidney.

	Time	Cor- rected urine volume per minute	Urea N	Blood urea N	Clear- ance in per cent of nor- mal
	minutes		mgm.	mgm.	
Denervated kidney...	30	1.75	121.8	17.09	17.5
Control kidney.....	30	1.79	142.5		20.6

On June 18, 1934, about seven weeks after the first operation, denervation was performed on the left kidney. The kidney measured 13 x 7 x 5.8 cm. in diameter. The exposure was difficult, and the operator was not satisfied with the completeness of the excision of the nerves about the renal artery.

The urea clearance rose after the second operation, reaching a maximum of 78 per cent of normal within three months. The ability to concentrate urine as measured by the nonprotein specific gravity rose from 1.014 to 1.023. Plasma proteins increased to a maximum of 5.7 per cent, and the protein in the urine decreased moderately. Blood pressure also increased somewhat, but appears now to be falling.

A very severe infection of the throat with hemolytic streptococcus apparently interrupted the trend toward increasing renal efficiency. Two months after operation lipids had diminished to the following values: total fat 1561 mgm., total cholesterol 442 mgm., lipid amino-nitrogen 6.9 mgm., total lipid nitrogen 23.4 mgm., lipid phosphorus 17.4 mgm. per 100 cc. of plasma.

Case 5. (See Figure 7.) Hospital Number 8295. (D.M.), aged 9 years. Onset of the nephritis occurred four months before admission during the course of a febrile disease, probably scarlet fever. The child had no complaints except for almost continuous edema of the ankles and often of the face.

Physical examination showed that there was moderate edema of the face, sacral region and shins. The lymph glands of the left submaxillary region were enlarged and tender. Blood pressure was elevated to 148/72 mm. Eyegrounds were normal. Many red blood cells and casts were found in the urine. It boiled almost solid with protein.

Hemolytic streptococci were grown from a culture taken from her throat. Two weeks later her temperature rose, and the cervical glands became acutely inflamed. Surgical drainage yielded pus from which hemolytic streptococci were grown. When this infection had cleared the hematuria rapidly subsided, but as many as 6,200,000 casts, of which 80 per cent were hyaline and 18 per cent granular, were excreted in 12 hours. Doubly refractive globules were found.

The urea clearance was 53 per cent of normal, maximum nonprotein specific gravity 1.018, and hemoglobin 13.4 volumes per cent oxygen capacity. The excretion of protein in the urine varied from 10 to 14 grams in 24 hours.

Edema disappeared with rest in bed and a light diet moderately low in salt. After discharge from the hospital three months later, edema again appeared. She was seen nine months later. There was slight edema of the shins, blood pressure was higher, the heart was moderately enlarged, and the retinal arterioles appeared somewhat constricted. The urea clearance had fallen to 34 per cent of normal. Two weeks later lobar pneumonia Types X and XXIX developed. The temperature was normal two days after onset, but complete resolution did not occur until after seven weeks. Gross hematuria developed, and the clearance fell to 25 per cent of normal.



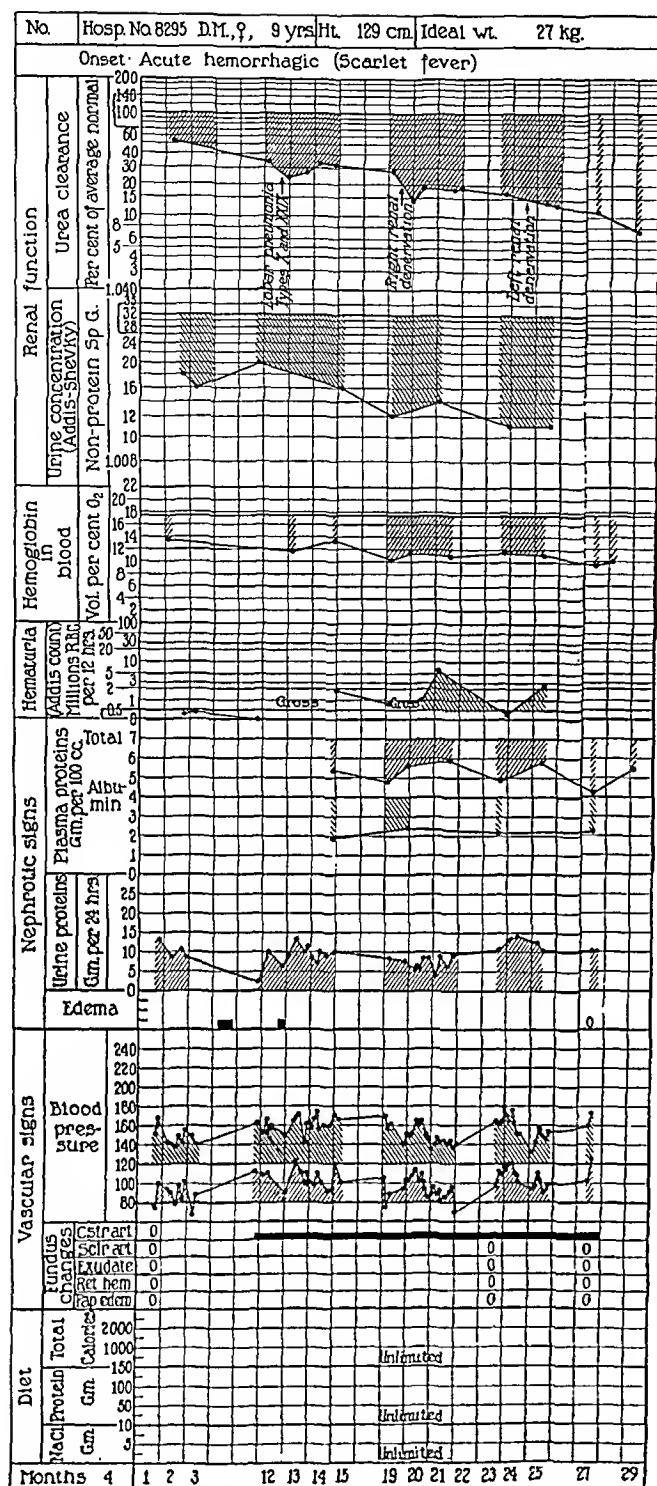


FIG. 7. BILATERAL DENERVATION IN PATIENT WITH CHRONIC ACTIVE NEPHRITIS.

One month after onset of pneumonia cardiac decompensation developed.

The prognosis appeared hopeless since the renal efficiency, as measured by the urea clearance and ability to concentrate urine, was steadily diminishing. Hemoglobin also fell progressively, and the blood pressure remained high.

On May 17, 1934, during the twenty-third month of the disease, right renal denervation was performed. The kidney was of the large white variety measuring 12 x 6 x

4.2 cm. The renal vessels appeared of normal size and showed no evidence of sclerosis.

Following operation the clearance and hemoglobin continued to fall slowly. Plasma proteins rose sharply from 4.89 per cent to 5.65 per cent and finally to 5.95 per cent in two months. The blood pressure was not altered significantly. Five months later on October 22, 1934, left renal denervation was performed. The kidney in size and color resembled the right. During the three months of observation following, the clearance continued to fall, as well as the ability to concentrate. Proteinuria continued, and blood pressure remained elevated.

#### Examination of blood vessels of muscle

At operation, specimens of lumbar muscle were removed for microscopic examination of blood vessels. Dr. C. P. Rhoads has given as his opinion that the vessels of only one patient (Number 2) exhibited definite evidence of intimal thickening. Case 5 also had slight morbid changes of the vessels; but the remaining patients showed no definite alteration.

#### DISCUSSION

Renal denervation had no immediate effect on renal efficiency, as measured by the urea clearance test. Van Slyke, Rhoads, Hiller and Alving (66) have found that in dogs the urea clearance parallels chiefly the renal blood flow. If the same parallelism occurs in nephritic patients, failure to increase the clearance indicates failure to increase the renal blood flow. The observation that clearances performed on specimens collected from the ureter of the denervated and innervated kidney of the same patient are practically alike, demonstrates in a conclusive manner the non-effect of denervation on the urea clearance. Nor did the specimens from the two ureters show any effect of denervation on the urine volume. Over a period of months following operation, the clearances of four patients have shown no tendency to change in a way other than that which might have been anticipated from the previous trend. One case (Number 4) differed in that it rose one month after the last denervation.

Rhoads, Van Slyke, Hiller and Alving (38) found no change in the clearance following denervation of normal dog kidneys. Page and Heuer (63) showed that denervation of the kidneys of a patient suffering from essential hypertension did not alter the clearance. The kidneys of this patient were normally efficient as measured by the clearance and concentrating ability.

Denervation caused neither increased urine flow nor diminished ability to concentrate, although animal experiments in the literature (11, 12, 13, 14, 15, 16, 17, 18) led us to expect these changes. On the contrary, two patients (Cases 1 and 4) exhibited a significant increase in concentrating power during the months after operation. In the remaining three cases a slow loss of this capacity occurred, parallel with reduction in the urea clearance. The loss in concentrating power was, however, no more rapid than is likely to occur during the usual progress of cases of this type (e.g., see charts of Cases 9 and 11 of Alving and Van Slyke (68)).

Excretion of red blood cells in the urine was sharply diminished in one case (Number 2), falling from one hundred million cells to slightly more than one million in a concentrated 12-hour specimen. It was uninfluenced in the other cases, in which excretion was already minimal. Excretion of protein was reduced in four of the five patients.

The most marked effect was observed in Case 1. Excretion of protein in the urine had been constantly at a level of 3 to 5 grams in 24 hours for a period of at least 16 months. Directly following *unilateral* denervation it fell to less than a gram and has remained so for 7 months. Proteinuria was diminished from an average level of 10 to 15 grams to 5 grams in Case 2, from 10 grams to 5 grams in Case 3, from 12 grams to 8 grams in Case 4, and was uninfluenced in Case 5.

A rise in plasma protein might have been expected as a result of decreased excretion in the four cases cited above. It is, however, difficult to tell whether the operation affected the plasma protein level. In Case 4 a rise from a preoperative level of about 4.5 per cent of total protein to 5.5 per cent occurred, but not until four months after the second denervation. In Case 3 there may have been a similar rise. However, it is common for similar rises to occur spontaneously, especially when the urea clearance falls to about 20 per cent of normal (See Alving and Van Slyke, Cases 6 to 11 (68)). The most that one can say definitely is that denervation does not lower the plasma proteins, and may assist in raising them.

As a result, probably, of the increase in plasma proteins, edema disappeared in Case 4, in which it had been present before operation. Following

the first denervation, it disappeared in a dramatic fashion, and, except for a slight recurrence for a period of a week, has not returned, in spite of the fact that salt was increased to 9 grams per day. Of the other patients, Numbers 1, 2, and 5, were edema-free before operation and have remained so. Number 3 showed sporadic edema both before and after.

Plasma lipid concentration was studied in two cases (Numbers 2 and 4). Reduction in all fractions was observed following denervation. It is difficult, however, to be certain that this was not a spontaneous change.

Blood pressure fell in all of the cases and remained below preoperative level for from weeks to months following each operation. The fall was not permanent, however; in each case the pressure later returned to, or near to, its former level. Experiments performed by Page (48) on dogs, in which hypertension was produced by x-ray irradiation of the kidneys or by constricting the renal arteries (49), showed that hypertension resulted in animals with their kidneys denervated as surely as in normal animals. There was, therefore, little reason to hope that denervation in patients would permanently reduce their blood pressure. Furthermore, it has been shown by Page (75) that renal efficiency, as measured by the clearance, is dissociated from the level of arterial blood pressure in patients with hypertension of the nephritic or essential variety. The probability may be assumed that renal blood flow also is not directly dependent on the level of arterial blood pressure.

From clinical observations of the weight and response of the patients to ingestion of water, we have no reason to believe that denervation alters the water balance of the body. It is reasonable, therefore, to assume that the extrinsic renal nerves do not control its water balance.

Our cases were selected from those in which the prognosis was bad and, further, those in which the morbid process had become chronic. Possibly if the operation were performed early in the course of the disease, more favorable results might be achieved. This is a matter on which we hope to report at a later date.

#### CONCLUSIONS

1. Renal denervation in patients with chronic nephritis caused excretion of protein to diminish

in four out of five cases. Unilateral denervation may be as effective as the bilateral operation in this regard.

2. Renal efficiency, as measured by the urea clearance test, is not altered following unilateral or bilateral denervation. It appears probable, from this result, that denervation does not increase renal blood flow.

3. Decreased ability to concentrate urine did not occur, although from animal experiments in the literature it might have been anticipated. On the contrary, two of five patients exhibited increased concentrating power.

4. The level of blood pressure fell in all of the cases for a few weeks after operation, but in all but one case regained its original value. Failure of denervation to affect the blood pressure appears to be evidence against the theory that nervous impulses, originating in the kidneys and conducted by their extrinsic nerve supply, are responsible for the genesis of hypertension in patients with chronic nephritis.

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# ON THE BEHAVIOR OF *HEMOPHILUS INFLUENZAE* IN CERTAIN DISEASES OF CHILDREN

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Dochez, Mills and Kneeland (2) have shown that on infection with the virus of "common cold," apes which had previously harbored a few *H. influenzae* in their throats may yield heavy cultures of this organism from both throat and nose. With the spread of distribution there was a change in cultural and immunological characteristics such that strains isolated during the infection fell within the category designated by Pittman (6, 7) as the smooth or fluorescent variant of *H. influenzae*. It seems that strains of this kind are responsible for most cases of influenzal meningitis, and that, as compared with the more familiar forms, they are characterized by a superior viability in the blood and tissues of experimental animals. "Fluorescent" strains may be recognized by their mucoid fluorescent growth on suitable media, by the demonstrable capsule and by the exhibition of a specific soluble substance which can be detected immunologically.<sup>1</sup>

It seemed desirable to supplement recent studies on the distribution of *H. influenzae* in the human upper respiratory tract (4, 13) by a consideration of the spread of this organism, both within the upper respiratory tract and elsewhere, with especial reference to the occurrence of fluorescent strains.

The nomenclature of influenzal variants is a vexed question. Since a distinct rough variant has been described (3, 10), the use of the term R for ordinary strains of influenza bacilli (6) seems likely to lead to confusion. We would suggest, therefore, the adoption of the terminology proposed by Dawson (1) for the pneumococci. In this paper, we shall designate fluorescent strains of *H. influenzae* as M (mucoid), and ordinary strains as S (smooth), reserving R for the rough variant.

## METHODS AND MATERIAL

The Pediatric Service at the New Haven Hospital consists of 60 beds (including a contagious

<sup>1</sup> By means of precipitation or agglutination tests these strains may be classified in six serological groups designated A to F.

division) to which are admitted patients under the age of 13 suffering from all types of acute non-surgical diseases. Nose and throat cultures are taken on all patients on admission and are repeated irregularly.

The nose and throat swabs are streaked on separate blood agar plates, and if the illness is thought to be respiratory, the swabs are usually shaken up together in 1 cc. of broth, which is then injected into the peritoneal cavity of a mouse. If the mouse dies or is killed, subcultures are made of the peritoneal exudate and heart's blood. Aural and other purulent discharges are invariably cultured, and blood cultures are taken as indicated. Blood and spinal fluid cultures are made within 20 minutes of death on all children who have died on the service.

In addition to the wards, there is an active outpatient clinic from which many cultures come to the laboratory.

All plates are carefully examined in oblique light for the small translucent nonhemolytic colonies of influenza bacillus.

Certain gram-negative diplococci give small colonies which may be confused with those of the influenza bacillus, especially if the plates are examined at 24 hours. At 48 hours such colonies are usually larger and more opaque than the influenzal colonies. Some of these diplococcus strains have been subcultured. These did not require the accessory "V factor" of Thjötta and Avery (11). Small non-hemolytic or slightly green streptococci may form colonies readily mistaken for those of Pfeiffer's bacillus. If there are only a few colonies of *H. influenzae* on a plate, one may be unable to get a satisfactory smear, and, if mixed with other common organisms, influenza bacilli will usually be outgrown on subculture. Hence an accurate estimate of the prevalence of *H. influenzae* is hardly possible.

Certain criteria of the species *H. influenzae* are given below.

Growth on blood agar plate	Morphology	Growth on special media	Growth requirements
Small, trans- lucent colonies. No hemolysis.	Gram-negative rods and cocco-bacilli.	More luxuriant than on ordinary blood media.	Requires both X and V factors (11, 12).

From February to December, 1933, only strains which promised to be interesting from their predominance or from their location in the nose, or other unusual site, were tested for fluorescence. After some checking with Dr. Pittman, we came to feel considerable confidence in our recognition of fluorescent (M), borderline ( $\pm$ ) and ordinary (S) strains. Unfortunately, a number of M strains obtained in this period were not typed. From December 23, 1933, to March 17, 1934, we attempted to study all strains systematically. All were subcultured on special media and on blood agar plates, and strains were kept on blood agar slants, transfers being made every 9 to 10 days. Dr. Pittman has kindly typed most of our M strains for us. All strains obtained in this latter period, except those lost on subculture, were subjected to the growth requirement test (11).

The technique used for this test, with slight modifications, was that communicated to us by Pittman (9) but Savita broth was used as a basic medium. The X factor was prepared by hemolyzing rabbit red cells with ether. The cells, thrice washed, were mixed with saline and ether in the proportion 10:10:4. The container was capped to prevent evaporation and kept in the icebox for 2 days. The rubber cap was then replaced by a cotton plug, and the tube placed in the incubator overnight to allow the ether to evaporate. The material was then centrifuged, and the solid residue discarded. The supernatant fluid, kept as stock in the icebox, was found to be sterile.

For the V factor about 500 cc. of unwashed brewer's yeast was mixed with 2,000 cc. of tap water. The mixture was heated slowly in an open pan with frequent stirring. When the temperature reached 80° C., the suspension was quite homogeneous. It was filtered through filter paper and through Berkefeld N candles.

To perform the test, 4 tubes were set up as follows:

A	B	C	D
2 cc. broth + 0.05 cc. 1/100 dilution of X factor + 0.1 cc. yeast extract (V).	2 cc. broth + 0.05 cc. 1/100 X factor.	2 cc. broth + 0.1 cc. yeast extract (V).	Same as A.

The tubes were incubated overnight to rule out contamination. A small portion of a single colony was fished with an inoculation needle into Tube A. The rack was then incubated for 24 hours. At this time *H. influenzae* would have grown well in A. A small loopful from this tube was then passed into each of the other three, and the rack was again incubated for 30 to 48 hours. Smears were made from Tube D. Tubes B and C should show no growth, if the organism was *H. influenzae*.

In our hands the "accessory factor" broth (X-V broth) has supported a more luxuriant growth than Levinthal (5, 9) or blood broth.

For reading fluorescence we have used both Levinthal agar (5, 9) and a medium prepared from the test factors (X-V agar). The latter may be made as follows: To 80 cc. of meat infusion broth add 20 cc. of yeast extract and 0.3 cc. of undiluted X factor. Mix the whole with 100 cc. melted 3 per cent agar, and pour plates. Such plates are satisfactory if used within 24 hours.

It has been our practice to streak each sector of a plate heavily with one of the strains to be tested. Plates have been read at 24 hours, each batch being controlled with a known fluorescent strain. The plate should be viewed by transmitted light against a dark background, and the opaque fluorescent appearance of a good M strain is striking. All influenza bacilli grew well on these media, but the growth of S strains is relatively meager compared to that of the M forms, and the S colonies are translucent. Certain strains are characterized by a luxuriant opaque growth with little fluorescence. These resembled certain of our stock strains, the fluorescence of which has been lost on subculture, and were classed as  $\pm$  or intermediate.

If a stock mucoid strain has lost its fluorescence, one may sometimes restore it by inoculating a heavy culture into a mouse and killing the mouse



after 12 to 24 hours. It will be noted that M strains have sometimes been obtained on mouse culture only. However, the survival of *H. influenzae* in the mouse is irregular, and the death of a mouse inoculated from nose and throat swabs is seldom due to Pfeiffer's bacillus alone. The mouse is not a satisfactory medium for the routine culture of *H. influenzae*.

#### RESULTS

As an estimate of the frequency with which mucoid variants may be found in the upper respiratory tract, we give the figures from the latter part of this study (December, 1933, to March, 1934).

Children studied ..... 186  
 Influenza bacilli found in upper respiratory tracts of 43  
 Mucoid variants found in upper respiratory tracts of 4  
 Intermediate variants found in upper respiratory tracts of 8

Thirty-four of the 67 strains studied, including all the M strains, were subjected to the growth requirement test. A number of organisms not thought to be *H. influenzae* were also tested. In all instances the results were as expected. It would seem that *H. influenzae* can usually be identified by its morphology and by the typical colony on blood agar.

Data on the entire period of study, and on some cultures taken before and after that period, are given in the tables. Material from 29 patients, who showed no M or intermediate variants in their upper air ways but had S forms in nose or ear, is summarized in Table I. Table II is based on a study of 11 children with intermediate  $\pm$  strains in their upper respiratory tracts, while the 13 who had mucoid strains are reported in Table III. Table IV includes four patients who had influenza bacilli in sites other than the upper respiratory tract; these strains were all mucoid. One of these children (K.L.) had influenzal meningitis. Another (R.C.), with otitis media, appears also in Table I. This child had one positive blood culture only and made a complete recovery. A third, Eng., 6 years of age, died outside the hospital one hour after the onset of laryngitis. The strain isolated from the heart blood at autopsy was kindly given us by the Pathology Department. In the pleural fluid of the child

R.F. tubercle bacilli were demonstrated by guinea-pig inoculation.

#### DISCUSSION

Pittman has emphasized (7) the extreme instability of freshly isolated M strains. It is possible that mucoid variants may dissociate or be overgrown during the first transplant, and that the use of media suitable both for primary isolation of *H. influenzae* and for demonstration of fluorescence might give different results from those recorded here. On the translucent media which we have used the growth of *H. influenzae* is inhibited by the presence of green streptococci or pneumococci (9). Our statement of findings must include the proviso "with the techniques used." Our conclusions must also include the qualification "in children" for bacterial behavior in adults might be quite different.

Of the cases of patients with *H. influenzae* in their noses there are three which one might hesitate to class as infections of the respiratory tract. Yet the asthma of the child F (Table II) was probably on an infectious basis, the child E.G. (Table I) had a tuberculous otitis media (proved at autopsy), and M.B. had a severe oral infection. Some of the children were seen several times, with and without respiratory infection; Pfeiffer's bacillus, often present in the nose in respiratory disease was not found there apart from such disease. The view that *H. influenzae* may spread to the nose or ear during infections of the respiratory tract is supported by the present study. The instances given include cases of scarlet fever and of Type I pneumococcus pneumonia, as well as of "common cold." Hence it is difficult to interpret the significance of this spread. In many cases we feel sure that the influenza bacillus played no great part in the disease. We cannot be sure of its rôle in otitis media. Our figures indicate that a spread of the bacillus may occur without the appearance of mucoid variants.

It is interesting that all the strains obtained from "distant sources" (Table IV) were mucoid. This finding accords well with Pittman's observation of the superior viability of M strains in the blood and tissues of experimental animals (7, 8). However, except in the case of the patient K.L. with meningitis, we have no means of



TABLE I  
Isolation of *S* forms of *H. influenzae* from nose, ear, etc.†

Patient	Age	Date	Nose	Throat	Mouse	Ear	Diagnosis
C. A....	4 years	April 17, 1933	S	S	—		Lobar pneumonia (Pn. unclassified)
M. M....	1 year	February 7, 1933	S	?	—		Bronchiolitis
R. L....	6 weeks	January 27, 1933	S	?			Bronchitis
J. R....	14 months	February 7, 1933	S	?			Lobar pneumonia (Pn. unclassified)
J. V....	6 months	January 3, 1933	S	?	?		Bronchiolitis
P. de S...	7 months	March 19, 1933	S	?			Rhinopharyngitis
W. H....	5 months	September 16, 1933	S	?		—	Otitis media
E. G....	2 years	April 5, 1933	S	S	—		Tuberculous otitis media and meningitis
H. C....	4 weeks	March 23, 1933	S	?			Bronchiolitis
P. P....	6 years	February 3, 1934	S	—	—		Scarlet fever
K. A....	8 years	January 10, 1934	S	—			Strep. pharyngitis
Z. P....	1 year	January 1, 1934	S	—			Rhinopharyngitis
B. R....	2 years	March 9, 1934	S	—			Otitis media
S. D....	8 years	November 10, 1933	—	—	—	S	Otitis media
P. A....	2 years	March 12, 1934	S	—			U. R. I.
V. C....	5 years	March 14, 1934	S	—			U. R. I.
F. C....	9 months	March 4, 1933	S	?			U. R. I., tuberculous peritonitis
E. L....	3 years	April 5, 1933	S	?		—	Scarlet fever
R. L....	5 years	April 5, 1933	S	?			Scarlet fever
		April 25, 1933	S	?			
R. C....	3 months	December 23, 1933	S	—		S	Otitis media
		January 4, 1934	—	—			Improved
		September 16, 1934*	—	—			Gc. vaginitis
A. K....	2.5 years	April 21, 1933	S	?			Scarlet fever
		May 12, 1933	—	—			Well
		July 28, 1934*	—	?			Bacillary dysentery
J. M....	5 years	January 7, 1934	S	—	S		Lobar Pn. (Pn. unclassified), bronchitis
		August 2, 1934*	—	?			Fever undiagnosed
M. B....	9 years	April 23, 1933	S	?			Dental abscess
		April 29, 1933	S	?			Cervical adenitis. Rheumatic fever
		September 28, 1934*	—	—			Congestive heart failure
D. M....	3 years	November 19, 1933	?	—			Bronchiolitis
		December 12, 1933	S	S			
		December 19, 1933	—	—			Improved
P. J....	12 days	March 15, 1933	—	—			Bronchopneumonia (pneumococcus).
		March 16, 1933	S	—			
		March 24, 1933	—	?			
J. Mas...	6 years	January 9, 1934	S	—	—		Lobar pneumonia (Pn. I)
		April 19, 1934*	—	—			Mesenteric adenitis
A. D'A..	6 days	November 18, 1932	—	—			Fat necrosis
		November 30, 1932	—	—			
		December 21, 1932	—	—		—	Otitis media
		May 6, 1933*	—	?		S	Otitis media
E. K....	15 months	February 5, 1933	?	?	—		Bronchitis and bronchopneumonia
		March 7, 1933	S	?			
		March 15, 1933	S	?			
I. M....	3 years	January 13, 1933	—	?			Cooley's anemia
		June 20, 1933*	—	—			Cooley's anemia
		September 12, 1933*	—	—			Cooley's anemia
		February 18, 1934	S	—			+ U. R. I.

† S = *H. influenzae*, S form.  
? = *H. influenzae*, strain not tested.  
— = No influenza bacilli recovered.

\* = Readmission.  
U. R. I. = Upper respiratory infection.

assaying the importance of mucoid influenza bacilli in these patients. We may note that Pittman has found that most of her meningeal strains are Type B (7, 8), and that two of our patients had Type B bacteremia without meningitis.

#### CONCLUSIONS

(1) A spread of *H. influenzae* to the nose may occur in a variety of infections of the respiratory

tract. This organism is also frequently isolated from aural discharges.

(2) While mucoid variants are occasionally isolated from the upper respiratory passages, the spread of *H. influenzae* to the nose or ear is frequently unassociated with the appearance of such a variant. No correlation is evident between the occurrence of M forms and the type and severity of the disease.

TABLE II  
*Isolation of strains intermediate between S and M forms*

Patient	Age	Date	Nose*	Throat	Mouse	Ear	Diagnosis
	years						
A. R. ....	10	May 16, 1932	—	?			Diabetes
		February 27, 1933*	S	—	±		Diabetes and U. R. I.
B. H. ....	1	December 2, 1932	?	?	?		Lobar pneumonia (Pn. XIV)
		March 3, 1933*	±	—		S	Otitis media
		March 9, 1933					
		July 9, 1933*	—	?			Lobar pneumonia (Pn. unclassified)
J. F. ....	1	October 10, 1933	—	—	±		Bronchiolitis, bronchopneumonia
		December 18, 1933	S	—		—	
		December 25, 1933					
		January 2, 1934	—	?			
W. S. ....	4	December 10, 1933	—	?			Lobar pneumonia (Pn. I)
		February 8, 1934	±	?			Empyema, pharyngitis
Kn. ....	1	January 12, 1934	—	—	±	S	Lobar pneumonia (Pn. I), otitis media
P. ....	2.5	January 25, 1934	S	—			U. R. I.
		February 10, 1934	±				
Ra. ....	1	January 28, 1934	S	—	—	±	Otitis media
C. R. ....	2.5	September 3, 1933	?				U. R. I.
		February 8, 1934*	±	—			Tonsillitis, adenitis
F. ....	3	February 23, 1934	S	—			Asthma
		March 2, 1934*			±		Asthma
Del. ....	1	March 5, 1934	±	S	—		Pharyngitis
D'Ag. ....	(0.4)	March 12, 1934	±	±	±		Bronchopneumonia

\* ± = Intermediate form of *H. influenzae*. For other symbols see Table I.TABLE III  
*Isolation of M strains of H. influenzae from upper respiratory tract*

Patient	Age	Date	Nose*	Throat	Mouse	Ear	Diagnosis
	years						
R. W. ....	1.7	February 14, 1933	—	M			Laryngitis
E. B. ....	8	February 23, 1933	M(B)	?	?		Scarlet fever
A. C. ....	1.3	March 27, 1933	?	?			Scarlet fever
		April 15, 1933	M(E)	M(E)			
R. A. ....	3	January 10, 1932	—	—			Lobar pneumonia (Pn. IV)
		September 19, 1932*	—	?			Bacillary dysentery
		November 23, 1932	?	?	?		U. R. I., anemia
		January 7, 1933*	—	?	—		Lobar pneumonia (Pn. IV)
		January 13, 1933	M(E)				
		January 27, 1933	—	?			
		December 25, 1933*	—	—	M(E)		Lobar pneumonia
		March 14, 1934*	—	S			Rhinopharyngitis, cervical adenitis
L. B. ....	4	March 24, 1934	S	S	S		
		January 17, 1933	—	S			Thyrototoxic goiter
		September 18, 1933*	M	?	?		Thyrototoxic + pharyngitis and tonsillitis
		September 21, 1933	M	?			
		September 27, 1933	M	?			
		September 30, 1933	M	?			
A. S. ....	1.5	October 12, 1933	—	—	M		Bronchopneumonia
S. R. ....	4.5	February 24, 1933	—	?			Acidosis
		March 2, 1933	—	?			
		September 15, 1933*	M	?	—		Tonsillitis
E. V. ....	8	October 17, 1933	M	?	—		U. R. I., epilepsy
W. K. ....	4.5	November 17, 1933	?	M			Pharyngitis
P. T. ....	3	December 17, 1933	—	—	M(A)		Epilepsy, U. R. I.
Bi. ....	2	December 28, 1933	—	S	M(E)		Lobar pneumonia (Pn. V)
R. F. ....	1.4	January 23, 1934	—	—			Tuberculous effusion
		January 25, 1934	—	—			Pharyngitis
		January 29, 1934			M(E)		
		February 21, 1934			—		
Cd. R. ....	2	March 6, 1934		M(A)			Follicular tonsillitis
		March 12, 1934	M(A)	M(A)			Improved

\* M(A) = Mucoid (fluorescent) *H. influenzae*, Type A (Pittman).M(B) = Mucoid (fluorescent) *H. influenzae*, Type B (Pittman).M(E) = Mucoid (fluorescent) *H. influenzae*, Type E (Pittman).

For other symbols see Table I.

TABLE II  
*Period of sensitization*

Number of days . . . . .	7	8	9	10	11	12	13	14	15	16	17	18	22
Number of rabbits . . . . .	2	2*	2	3	4	5	0	1*	1	1	4	2	1

\* See footnote to Table I.

injections were made, was the measure used in estimating whether or not skin sensitivity had been produced. Two diameters of the resulting wheals were recorded in centimeters but no attempt was made to measure accurately the oedema or assign degrees of intensity to the erythema produced. (Table III.)

TABLE III  
*Twenty-four hour readings*

Average size of first reactions in all animals. . . . . 2.0×1.6 cm.  
Average size of last reactions in all animals. . . . . 3.5×2.8 cm.  
Average size of smallest reactions in all animals. 1.4×1.2 cm.  
Average size of largest reactions in all animals. 4.1×3.1 cm.

Since the number of organisms introduced into the skin at each injection was relatively large, the contrast between the size of the first and last wheals produced was not as great as it might have been had smaller doses been employed. Sensitivity, as reflected by the size of the skin reaction, did not always mount in a straight line. The initial response was often greater than some reactions produced by subsequent injections. After the first response the wheal would often increase beyond the original level only to diminish in size or in some instances to increase again if further injections were made. In general, there were three criteria by which an animal was considered to be sensitized. (1) If it had received three or preferably more injections which had produced wheals greater in diameter than one centimeter. (2) If it had shown a maximal reaction following some one of the injections after the first. (3) If the last injection was followed by a reaction larger than one centimeter in diameter and definitely larger than the original one.

When an animal had received repeated skin injections and was considered sensitive by the criteria just given, a heat killed culture of the streptococcus (SD<sub>2</sub>) employed was injected directly into the pericardial sac. A twenty-four hour culture of SD<sub>2</sub> in beef infusion broth of pH 7.6 was killed by heating it in a water bath at 56° C.

for one hour; sterility was then demonstrated by cultures. The organisms were not resuspended in salt solution before pericardial injection.

After the final sensitizing injection a period of from one to seven days elapsed before pericardiotomy was performed. (Table IV.) Under

TABLE IV

*Days elapsing between last injection and operation*

Number of days . . . . .	1	2	3	4	5	6	7
Number of rabbits . . . . .	6	3	6	8	0	2	3

ether anaesthesia the thorax was opened and the parietal pericardium was picked up in small forceps. In this way the operator could be reasonably certain that the inoculum was introduced accurately into the pericardial sac. Two cc. of the killed culture were introduced into each animal in the series, both into the controls as well as the sensitized animals. Three animals, previously sensitized, died within an hour after operation and were discarded. None of the control animals died. The majority of the sensitized animals were sacrificed on the third day after operation. A few were sacrificed sooner and a few were kept for as long as six days. All of the controls were sacrificed on the third day. A characteristic protocol follows.

#### PROTOCOL 1

White rabbit number 41

*A. Sensitization:* Uniform doses of 0.1 cc. saline suspension of twenty-four hour cultures of living beta haemolytic streptococcus (SD<sub>2</sub>) were injected intracutaneously.

Dose	Date given	Twenty-four hour reaction
1 . . . . .	March 26, 1932	1.5 cm. × 2.4 cm.
2 . . . . .	March 28, 1932	1.4 cm. × 1.5 cm.
3 . . . . .	March 30, 1932	2.0 cm. × 2.5 cm.
4 . . . . .	April 2, 1932	2.0 cm. × 2.5 cm.
5 . . . . .	April 7, 1932	3.5 cm. × 5.5 cm.
6 . . . . .	April 10, 1932	2.5 cm. × 3.0 cm.

*B. Operation:* April 13, 1932. Under ether anaesthesia open pericardiotomy was performed and 2.0 cc. of vaccine prepared from beta haemolytic streptococcus (SD<sub>2</sub>) was injected into the pericardial sac.

*C. Autopsy:* April 16, 1932. The animal was sacrificed and the thorax opened. The parietal pericardium was opaque and covered with a thick fibrinous exudate. The pericardial sac was greatly distended with an excess of opaque fluid which gave no growth on culture. Direct



FIG. 1.  $\times 100$ . CONTROL ANIMAL.

No skin injections. 2 cc. SD<sub>2</sub> vaccine intrapericardially. slight reaction in epicardium, myocardium normal.

means of this fluid showed masses of cells about half of which were polymorphonuclear leukocytes and about half small lymphocytes. No organisms were seen in the fluid with appropriate stains. The parietal pericardium was greatly thickened and covered with a dense grayish exudate. The heart seemed to be dilated. The valves appeared normal. No thrombi were seen in the larger coronary vessels. Microscopical sections revealed an intense diffuse inflammatory reaction involving the pericardium and both the ventricular and auricular myocardium.

#### RESULTS

At autopsy cultures were made from the pericardial sacs of both series of animals. In only one instance was there a secondary infection and the organism recovered was a staphylococcus. This animal was discarded. The remaining cultures were sterile. In all of the control animals the hearts appeared grossly normal. In the sensitized animals the gross appearances at autopsy

varied considerably. In thirteen there was no abnormality. In seven there were slight deviations from the normal appearance and in eight there were extensive changes. In four of these there were quite large pericardial effusions. The pericardial surfaces were greatly thickened and were covered with heavy fibrino-purulent exudate. Direct smears from the fluid in those instances in which there were pericardial effusions showed about an equal number of mononuclear cells and polymorphonuclear leukocytes. No bacteria were seen in stained preparations of pericardial fluid. In the hearts which were grossly abnormal the larger coronary vessels were studied as carefully as their small size would permit. No thrombi were found. No gross abnormalities were detected on any of the heart valves.

Sections for microscopical study were made from the hearts of eighty-nine animals. Thirty-one normal, healthy young rabbits served as one control group. The tiny scars, areas of vacuolization of muscle fibers and minute collections of round cells appearing in the sections of hearts in this series were considered normal and served as a standard with which to compare other sections. Ten animals were sensitized by repeated skin injections, as previously described, and were then sacrificed, without further procedure. Microscopically all of these hearts were normal. A third, and the most important control group, consisted of twenty normal rabbits that received no skin injections whatever. By open pericardiotomy as described above they received 2 cc. of a killed culture of SD<sub>2</sub> directly into the pericardial sac and were sacrificed approximately seventy-two hours later. Sections from the hearts of fifteen of these animals were recorded as normal. In the remaining five there were lesions recorded as "slight" which will be discussed below.

The microscopical appearance of the hearts of the twenty-eight animals, sensitized before the intrapericardial injection of vaccine, are remarkably different from those of the controls. Eighteen of these show extensive abnormalities which far exceed those noted in any of the controls. Four show changes roughly comparable to "slight" changes noted in the control group (mentioned in the paragraph above). The remaining six are considered normal. In the eighteen hearts considered extensively diseased the le-

sions varied considerably both in character and in distribution. In some the entire heart was involved while in others the lesions were focal in distribution. In some hearts the pericardial surfaces were extensively diseased whereas the myocardium was relatively spared and *vice versa*. There follows a tabulation of the changes noted in the eighteen hearts considered extensively diseased.

Pericarditis, diffuse .....	15
"    focal .....	3
Myocarditis, diffuse .....	12
"    focal .....	6
Aortitis .....	5
(Aorta visible* .....	6)

\* The sections did not all include the aorta.

In most instances the pericardial membranes were greatly thickened and covered with dense fibrinous exudate. In the earliest lesions polymorphonuclear leukocytes occurred in considerable numbers but in most instances the cells were



FIG. 2.  $\times 100$ . SENSITIZED ANIMAL.

2 cc. SD<sub>2</sub> vaccine intrapericardially. Intense pericardial reaction with dense fibrin deposit infiltrated by relatively few small round cells. Haemorrhage. Connective tissue cells in great abundance. Invasion of inflammatory reaction into adjacent myocardium.

chiefly small round cells with little cytoplasm and uniform dark nuclei. It is worthy of note that the number of leukocytes was far less than one would expect in such an intense inflammatory reaction with tremendous outpouring of fibrin. Perhaps the most striking feature of the reaction was the tendency to the formation of scar tissue and the whole inflammatory reaction was densely invaded by young fibroblastic cells. In Figure 2

the epicardial reaction is shown, and as can be seen clearly the inflammatory changes extend deep into the adjacent myocardium destroying muscle fibers and bringing about cloudy swelling and loss of striation in the more normal fibers near the periphery of the myocardial lesions. Here also there is great invasion of young fibroblasts and rapid formation of scar tissue with destruction of the normal intracellular substance.



FIG. 3.  $\times 100$ . SENSITIZED ANIMAL.

2 cc. SD<sub>2</sub> vaccine intrapericardially. Focal epicardial reaction. Small round cells, giant cells, connective tissue cells, muscle necrosis.

While the majority of hearts from the sensitized group showed widespread pericardial changes, in some the pericardial reaction was focal in distribution and in others it was uniformly very slight. Figure 3 illustrates a focal epicardial lesion. There is necrosis of the underlying muscle fibers with many small round cells and numerous young connective tissue cells. As can be seen clearly in the illustration there are

many giant cells. In some of the hearts with relatively little pericardial reaction the myocardium was extensively altered. Frequently this myocardial change extended down to the subendocardium and in many there was extensive destruction of the papillary muscles. Figure 4 shows a subendocardial lesion, which is almost precisely like the epicardial lesion illustrated in Figure 3.

A different type of reaction is illustrated in

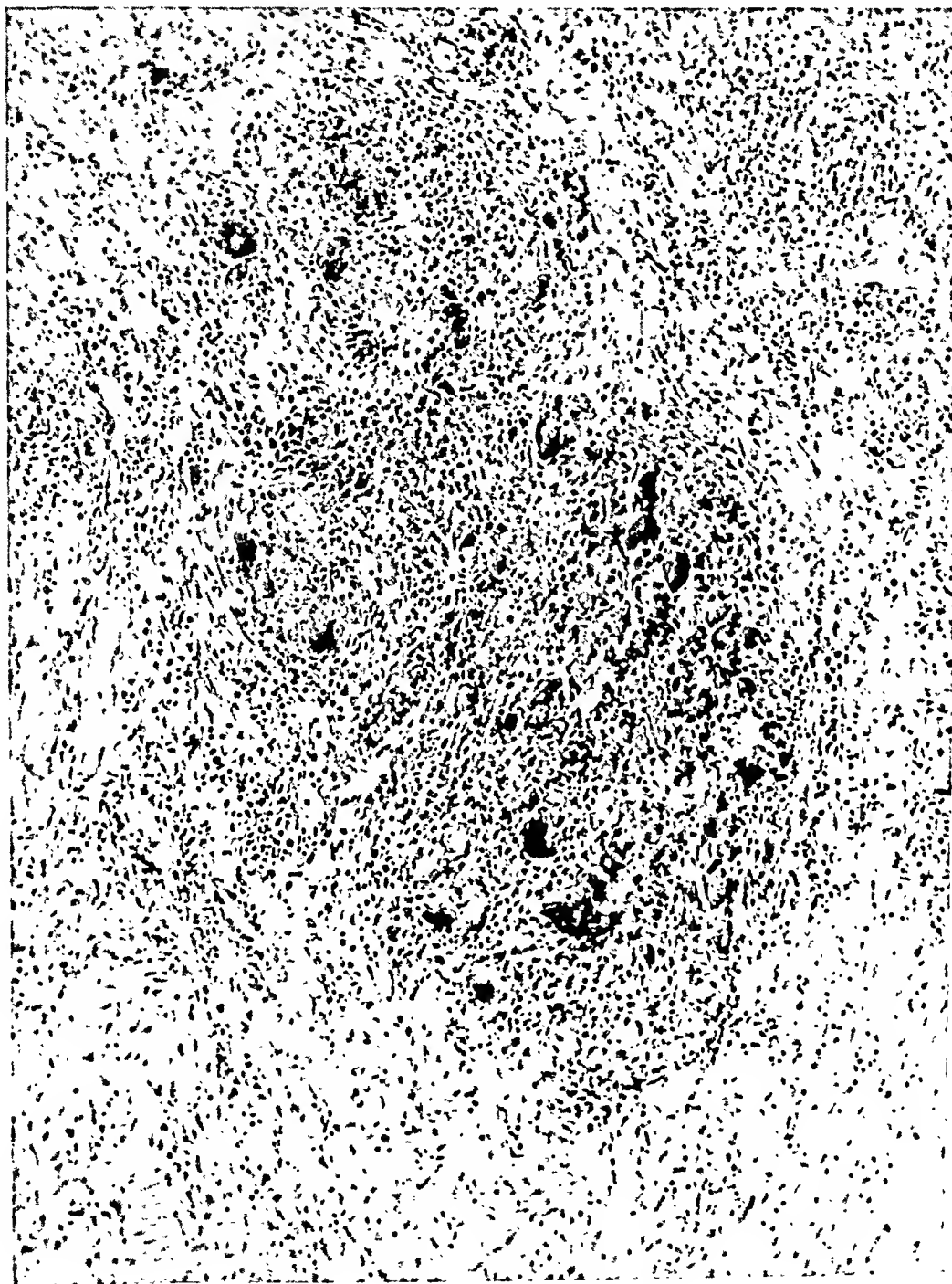


FIG. 4.  $\times 100$ . SENSITIZED ANIMAL.

2 cc. SD<sub>1</sub> vaccine intrapericardially. Focal reaction similar to Figure 3 located subendocardially.



Figure 5 in which a whole section of muscle from the depth of the left ventricle is almost completely destroyed. There is widespread necrosis of muscle with oedema, some haemorrhage, invasion of small round cells and fibroblasts.

Careful search of the blood vessels did not reveal any thrombi. In some of the sections the same type of inflammatory reaction was seen in the loose tissue about the aorta and in some the adventitia was likewise involved. None of the valves showed any significant change though

often the diffuse changes noted above involved the myocardium at valvular junctions. Careful examination of the vessels revealed some perivascular lesions but in comparison to other widespread changes they are rare.

#### DISCUSSION

When rabbits were made skin sensitive by repeated skin injections with living beta haemolytic streptococci and a vaccine prepared from the homologous organism was injected into the peri-



FIG. 5.  $\times 100$ . SENSITIZED ANIMAL.

2 cc. SD<sub>2</sub> vaccine intrapericardially. Widespread diffuse inflammatory reaction of myocardium, necrosis of muscle, oedema, haemorrhage, round and connective tissue cell invasion.



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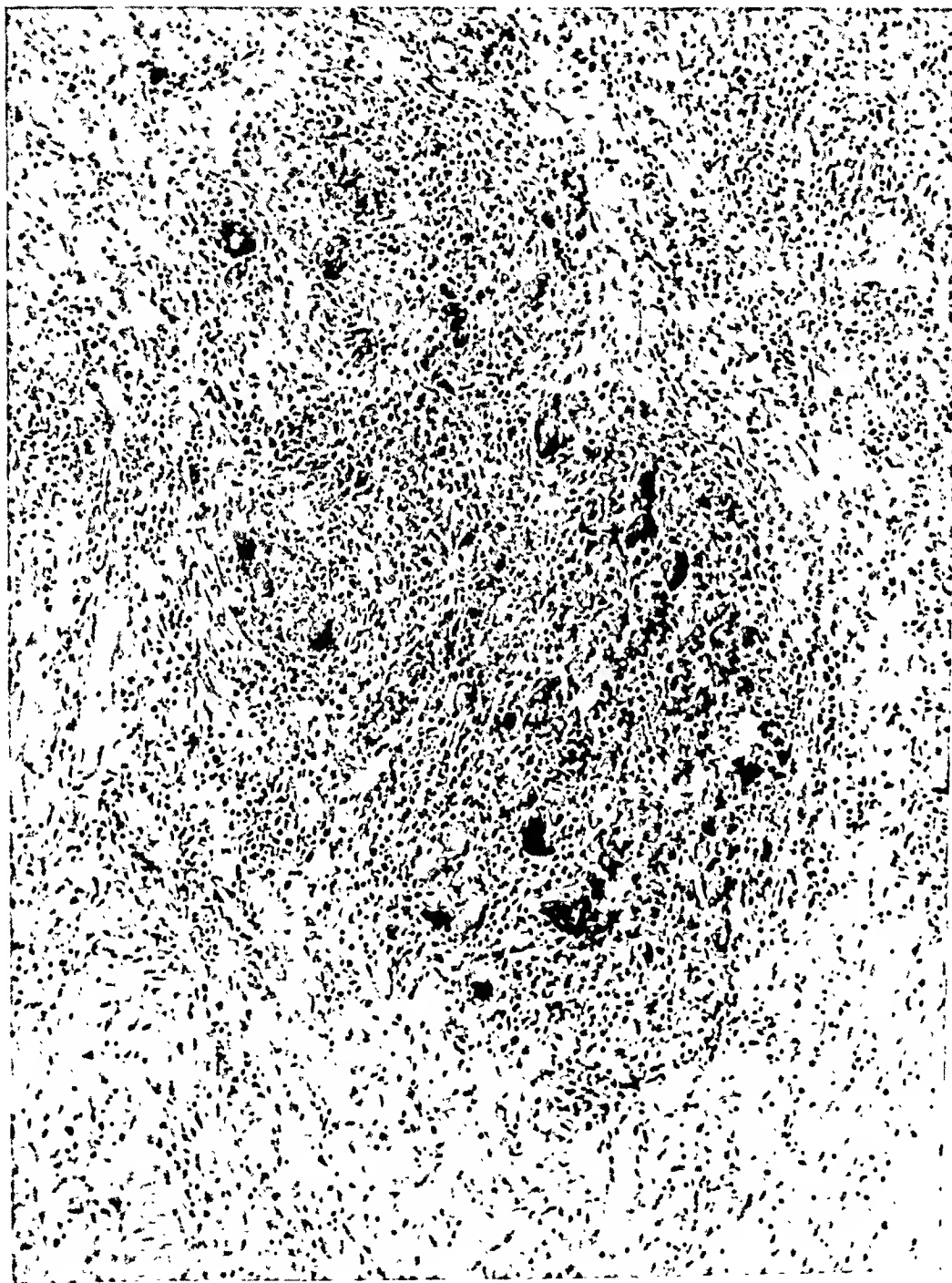


FIG. 4.  $\times 100$ . SENSITIZED ANIMAL.

2 cc.  $SD_2$  vaccine intrapericardially. Focal reaction similar to Figure 3 located subendocardially.

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# THE RATE OF ABSORPTION OF IODIDE AND GLYCINE FROM THE GASTRO-INTESTINAL TRACT IN NORMAL PERSONS AND IN DISEASE CONDITIONS

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Deficiency disease, other than that related to deficient dietary intake or to increased metabolic needs, may result from two kinds of disorder of the gastro-intestinal tract. First, the secretory mechanism, and secondly, the absorptive mechanism may be at fault. The present study is not concerned particularly with the first, an outstanding example of which is diminution or loss of the "intrinsic factor" from the stomach, which is responsible for Addisonian pernicious anemia. The absorptive mechanism may be at fault in a variety of ways, for example, when diarrhea is present, or when there are pathological changes, such as ulcerations of the bowel, infections, stricture or tumor, which may, by mechanisms not well understood, affect motility or produce mucosal changes (1, 2, 3).

The difference between the dose of iron or liver extract given orally and given parenterally to bring about optimal blood regeneration in certain types of anemia is very great. Absorption of these two substances obviously does not take place with great ease, although, in the case of the anti-pernicious anemia principle in liver extract, there may be some destruction of the substance in the stomach and bowel. When the mechanism of absorption is disturbed, clinical signs of deficiency of these substances might easily appear without the signs of deficiency of more readily absorbable substances, such as the products of protein, carbohydrate, and fat digestion.

For a number of years it has been felt in this clinic that some method for judging variations in the absorption rate from the gastro-intestinal tract would be of clinical value. This problem is fraught with many difficulties. The method employed in animals of observing the disappearance of a substance in the surgically isolated gut, or the analysis of the contents of the gut removed post-mortem, a method used by Cori (4) with success

and by many other observers in animal physiology, obviously cannot be used in human beings except under most unusual circumstances.

Study of the length of time between the ingestion of food and its appearance in feces, together with the character of the feces, which Burnett (5) employs as an indication of absorption is of some value but does not yield specific information. The upper small intestine is the organ chiefly concerned with the absorption of necessary elements of the food, and until more information is obtained, it cannot be concluded that the motility of the bowel as a whole or of the colon and rectum in particular, reflects the rate of absorption of materials in the upper small intestine.

Other objections apply to the study of the quantitative chemistry of the food and the feces. The fallacies of such a study appear when it is considered that the gastro-intestinal tract acts as a secretory as well as an absorbing organ. Moreover, food substances, readily detectable in the feces, may be absorbed well when the gastro-intestinal tract is obviously damaged, or they may not be demonstrated, because they are destroyed by enzyme or bacterial action.

These methods of judging the rate of absorption may be classified as direct methods. One must fall back on indirect methods to gain more information in man. The part played by the gastro-intestinal barrier has been frequently taken into account in studies involving the appearance of an ingested substance in the blood stream or in the urine, for example, in the study of glucose tolerance tests. In special circumstances, unusual methods have been employed, examples being the appearance of iodine in the sputum after the ingestion of an iodine salt (6), the transference of weight to distant parts of the body after the drinking of a measured quantity of water (7), the physiological effects of orally adminis-

Before it can be declared that the appearance time of iodine in the sputum after ingestion is a measure of the rate of absorption from the alimentary tract, it is necessary to take into account a number of factors. The emptying time of the stomach is undoubtedly important. Nevertheless, achlorhydria which was present in most of the patients studied, is usually accompanied by no great change in the emptying time, and the emptying time may be hastened (15, 16). A small amount of iodine undoubtedly is absorbed by the mucous membranes of the mouth (17) and of the stomach (18). A slow circulation rate of the blood probably would not delay appreciably the appearance time of iodine. Factors which regulate the level of the iodine of the blood, such as tissue storage or kidney or bowel excretion, may be important. The functional ability of the salivary glands is undoubtedly important.

To assist in ruling out these factors, potassium iodide was given intravenously, and the saliva was tested in the usual way. It was discovered at once, that relatively large amounts of potassium iodide (0.075 to 0.1 gram in 5 per cent solution) must be given in order to recover iodine uniformly in the saliva. This fact indicated that the 0.25 gram potassium iodide given by mouth must be rapidly and completely absorbed in normal individuals. The time of appearance of iodine in the saliva after the intravenous injection of 0.075 or 0.1 gram potassium iodide, varied between one and six minutes in sixteen subjects. In three subjects the time of appearance was seven, eight, and ten minutes, respectively. The time of appearance after intravenous administration could not be correlated with the time after oral administration. This fact indicates that variation in the appearance time after oral administration of a uniform dose of potassium iodide is dependent chiefly upon conditions in the gastro-intestinal tract.

To summarize: *the appearance time of iodine in the saliva after the ingestion of 0.25 gram potassium iodide is delayed in a wide variety of clinical conditions, including cases of anemia associated with achlorhydria. It is probable that this delay is a rough measure of disturbance of the absorptive ability of the upper small intestine.*

2. *The amino-acid nitrogen of the blood after the ingestion of glycine and Witte peptone.* The fasting whole blood amino-acid nitrogen averaged

for all subjects (4 normal individuals and 19 patients) 6.6 mgm. per 100 cc., the extremes being 5.6 to 7.8 mgm. per 100 cc. One exception was a value of 9.3 mgm. per 100 cc. in a case of lobar pneumonia during crisis. These figures correspond closely with those reported by others (19, 20, 21).

Average data for the blood amino-acid nitrogen after the ingestion of glycine or Witte peptone are presented in Figure 1. The rise of amino-acid nitrogen corresponded in general with that reported by Folin and Berglund (19) and Witts (21). The curves for patients resembled those for normal subjects. The amino-acid nitrogen reached the highest level usually in from 60 to 120 minutes after the ingestion of glycine. In some patients, the highest level was not attained until 180 minutes had elapsed, which is very probably abnormal. The most marked example of such a delayed "peak" was in a case of chronic nephritis with uremia. Also, in one case of myxedema, the "peak" was delayed, and in a second case of myxedema the 180-minute value was somewhat elevated. Witts (21) has reported high values of amino-acid nitrogen after glycine ingestion in uremia and myxedema.

On theoretical grounds, a delay in absorption should result in a lower 20-minute amino-acid nitrogen level. The rise of amino-acid nitrogen 20 minutes after the ingestion of glycine was similar in the patients and in the normal subjects, (Figure 1). It was the same in those subjects having a delayed iodine appearance time in the saliva as in those having a normal iodine appearance time. The average curves of the latter two groups are indeed strikingly similar.

The average curve for amino-acid nitrogen of the blood, after the ingestion of 25 grams Witte peptone in nine patients, is similar to the other curves in Figure 1, but shows a somewhat more rapid rise in the first twenty minutes and is of shorter duration. Witte peptone, containing mostly larger molecules than glycine, should theoretically be absorbed more slowly than glycine. The curve (Figure 2), however, might be taken to indicate that Witte peptone is absorbed even more rapidly than glycine. The proper interpretation is, probably, that the factor of absorption in the gastro-intestinal tract plays only a small part in determining the course taken by the amino-

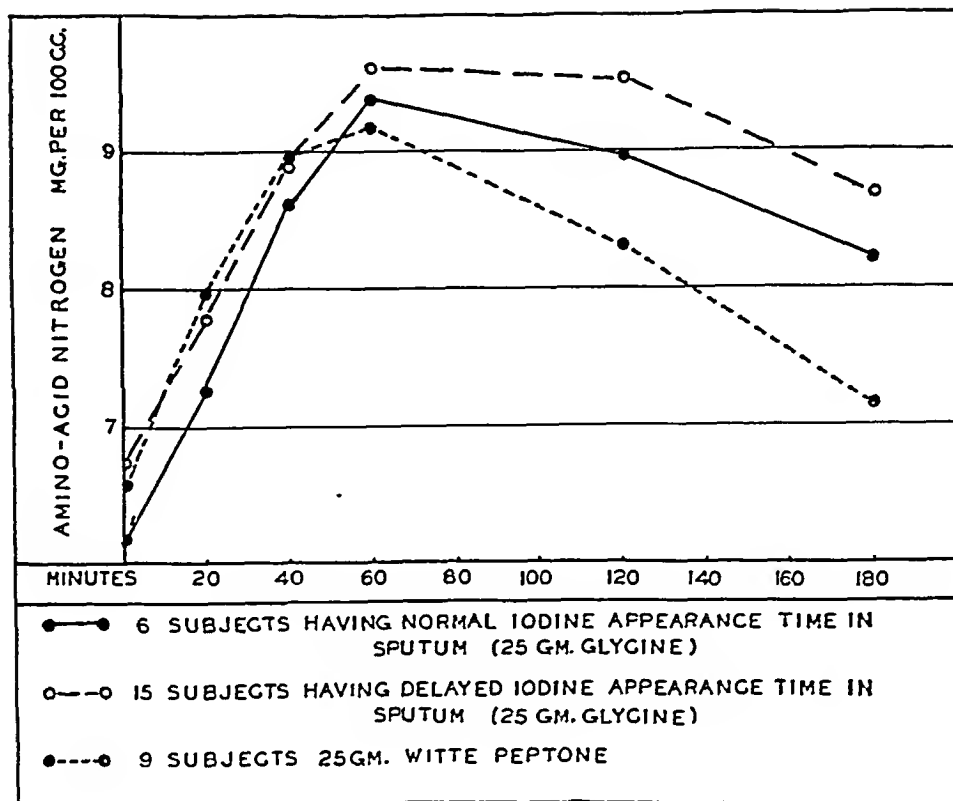


FIG. 1. AVERAGE DATA OF WHOLE BLOOD AMINO-ACID NITROGEN FOLLOWING THE INGESTION OF 25 GRAMS GLYCINE, AND 25 GRAMS WITTE PEPTONE.

acid nitrogen of the blood following the ingestion of glycine or of Witte peptone.

In seven patients 4 grams glycine in 10 per cent solution were injected intravenously. The injection was attended by no untoward symptoms. Blood samples were obtained at 4 or 5, 20, 40, 60 and 80 minutes. In all cases except one, the 4 or 5-minute value ranged between 10.1 and 12.3 mgm. per 100 cc. A rapid fall then occurred, and the fasting level was reached in 40 to 80 minutes. The injection of 4 grams glycine should theoretically result in an immediate rise of the blood amino-acid nitrogen to about 22 mgm. per 100 cc., if the blood volume is 5,000 cc. This illustrates how rapidly excess glycine can be removed from the circulating blood. About 75 per cent of the injected 4 grams glycine must be taken up by the tissues or excreted in the urine in the first 4 or 5 minutes.

A marked similarity in the curves obtained after oral and after intravenous administration of glycine was observed in two cases. In one, a

case of alcoholic cirrhosis, there was only a moderate rise in both instances; in the other, a case of chronic nephritis with uremia, there was an unusually high rise. The similarity of these curves suggests that the nature of the curve following oral administration of glycine depends more upon the character of the process of removal of glycine from the blood stream, than upon the rate of absorption from the gastro-intestinal tract.

The data presented here suggest that the study of the fate of glycine in the blood stream is without value in interpreting the absorptive capacity of the gastro-intestinal tract. Glycine is absorbed very rapidly, and any difference in the rate of absorption in different patients seems to be offset by the strong tendency of the regulatory mechanism to maintain a normal amino-acid nitrogen content of the blood. This regulatory mechanism is undoubtedly influenced by other factors which cannot be easily controlled. The most important factors are, probably, the previous state of nutrition of the subject, and the needs of the body at the

time. Martens (22) gives the opinion, that even the permeability of the intestinal mucosa to protein products obeys not only the composition of the intestinal contents, but also the factors of internal metabolism.

In summary, it may be said that *the amino-acid nitrogen content of the blood following the ingestion of 25 grams of glycine gives no useful information regarding the rate of absorption from the gastro-intestinal tract.*

#### COMMENT

It has been pointed out that numerous factors play a rôle in the normal absorptive mechanism. Undoubtedly several of these factors may be altered at one time by disease. It is possible that increased function of one factor may compensate for decreased function of another. It is probable that there occur selective hindrances to the absorption from the gastro-intestinal tract of certain classes of substances, other classes of substances passing the barrier in a normal fashion. The state of affairs being so complex, it would be hazardous to generalize about intestinal permeability from data regarding the absorptive rate of a single substance. The accumulation of much experience is required before definite conclusions regarding absorption can be made upon a given patient.

The study of the appearance time of iodine in the saliva after the ingestion of potassium iodide has shown some strikingly contrasting results in normals and in patients. One is strongly tempted to ascribe these changes to alterations in the absorptive rate of the small bowel, at least in respect to this particular substance. The administration of potassium iodide intravenously whereby the influence of the intestinal barrier is avoided, gives corroborative evidence which favors this conclusion.

It seems unfortunate, at least from the point of view of the study of the absorptive ability of the gastro-intestinal tract, that a substance like glycine which may be detected readily in the blood stream, offers so little information regarding absorption. The internal metabolism of glycine, its storage and disposal, seems to have much more influence upon the level in the blood stream than the rate at which the bowel delivers it to the cir-

ulation. The same seems to be true of glucose, and Trimble, Carey and Maddock (23) have stated that the concentrations of sugar existing in the venous blood may fail to reflect either the course or the completion of the process of glucose absorption.

Any procedure, however, which gives definite information about the function of absorption of the gastro-intestinal tract would be of considerable clinical value today. It would assist in elucidating problems of minor degrees of malnutrition which so frequently present themselves, and would aid greatly in the treatment of such conditions.

#### SUMMARY AND CONCLUSIONS

1. There is much evidence that nutritional deficiency may result from disorders of the gastro-intestinal tract which hinder absorption of essential food factors. A suitable test for the functional ability of the absorptive mechanism would be of considerable clinical value.

2. The appearance time of iodine in the saliva, after the ingestion of 0.25 gram potassium iodide, was delayed in a wide variety of clinical conditions, including patients who had achlorhydria or who suffered from some disease process likely to condition a disturbance of the absorptive mechanism. It is believed that this delay is a rough measure of disturbance of the absorptive ability of the upper small intestine.

3. The amino-acid nitrogen content of the blood, following the ingestion of 25 grams glycine, gave no information of value regarding the rate of absorption from the gastro-intestinal tract.

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# THE HEART IN THYROID DISEASE. I. THE EFFECT OF THYROIDECTOMY ON THE ORTHODIAGRAM<sup>1</sup>

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The observations which we wish to report have been made with two purposes in mind: to determine (1) what changes, if any, occur in the size and shape of the heart, as determined by orthodiagraphy, in patients with toxic and non-toxic goiter, and (2) the effect of partial or subtotal thyroidectomy, in the same subjects, upon the orthodiagram. Our material consisted of 102 patients with toxic goiters, and 35 with non-toxic goiters. The non-toxic group consisted of the nodular variety except for one case of carcinoma; several patients had diffuse enlargements of the gland in association with the non-toxic nodules. In a few instances the presence of hyperthyroidism was not easy to determine. Such cases were classified only after consideration and correlation of the history, physical findings, basal metabolism readings, and histologic appearance of the excised thyroid tissue. All patients were on the Thyroid Surgical Service of Dr. C. H. Frazier at the Hospital of the University of Pennsylvania and were studied through his courtesy. The operation consisted of subtotal thyroidectomy in 84 of the toxic patients (performed in one stage in 51 cases and in two stages or more in 33) and unilateral lobectomy in the remaining 18 (16 nodular and 2 diffuse). In the non-toxic group unilateral lobectomy was done in 23, and subtotal thyroidectomy in 12. The anesthesia in almost all instances was a combination of nitrous oxide and local, often with pre-anesthetic narcosis obtained by avertin.

The plan of study included—in addition to the usual history, physical examination, and routine blood and urine studies—electrocardiograms, orthodiagrams, and basal metabolism determinations made as soon as possible after admission to the Ward.<sup>2</sup> The latter three studies were repeated

within 7 days after operation and at intervals of about 3, 7, and 12 months thereafter. All three studies were usually made on the same day; when this was not possible, the interval between them did not exceed 3 days. In those patients of the toxic group who had multiple stage operations, follow-up intervals were dated from the last operation. Additional clinical studies were made when indicated. Thirty patients were followed for less than one year. Four toxic patients have been followed for over 2 years. Observations on the general physical condition with particular respect to the cardiovascular system and thyroid were made at each follow-up examination. All orthodiagrams were made by the same individual (A. M.).

## HEART SIZE

The literature concerning heart size in patients with goiter presents certain difficulties when attempts at comparison are made because of (1) varying classifications of thyroid disease; (2) different methods employed in measuring the heart and determining significant variations from normal in size; and (3) the inclusion of patients with associated conditions which might, *per se*, affect the size and shape of the heart.

Table I presents a review of the literature on heart size in goiter before and after thyroidectomy. Considering only those authors who classify their cases as toxic and non-toxic, it will be seen that the preoperative percentage of cases with cardiac enlargement varies from 26 to 83 per cent in the toxic cases and from 10 to 59 per cent in the non-toxic cases. Exclusion of groups with associated cardiovascular disease reduces the percentages in the toxic group to the range 26 to 32 per cent and in the non-toxic group to the range 10 to 14 per cent. The analysis of postoperative changes in heart size in the few satisfactory reports indicates that there is a tendency for hearts enlarged before operation to become smaller or remain unchanged in size, whether associated with toxic or non-toxic goiter.

<sup>1</sup>Read in Abstract before the Section on General Medicine, College of Physicians of Philadelphia, April 23, 1934.

<sup>2</sup>A number of patients were examined in the Outpatient Clinic before admission to the Ward, and these studies were included in the analysis.

TABLE I

*Summary of literature on heart size in patients with goiter before and after thyroidectomy*

Author	Number of cases	Associated cardiovascular disease	Goiter type and percentage of cases enlarged							Method *	Heart size—postoperative													
			Mechanical	Toxic	Mixed mechanical and toxic	Tracheal stenotic	Mixed stenotic and toxic	Neither stenotic nor toxic	Non-toxic		Adenoma	Hyperplasia	Cases	Follow-up period	Enlarged before operation			Normal before operation			Small before operation		Per-centage cases enlarged	
															Larger	Smaller	No change	Larger	Smaller	No change	Larger	Smaller	No change	Toxic
Kraus (1, 2)	?	?								P+T	?	months ?			Yes									
Blauel, Muller, Schlayer (3)	90	No	18	100	69					O	16	av. 12			16									
Steiner (4)	68	No	28	58	55					P+O	16	12			11									
Willius and Boothby (5)	377	?		most						P														
Coller (6)	300	?							18 to 50	P+O														
Kerr and Hensel (7)	181	?							33 74	P														
Meyer and Sulger (8)	125	?		33	73	39	78			T	64	24 to 36	4	7	27	10	0	16						
Meyer-Borstel (9)	156	?		83				59		?	?	?	some	0										
Hurxthal, Menard, Bogen (10)	200	Yes		46				41		T														
Parkinson and Cookson (11)	130	No ?		45						O+T	11	1 to 3	3	3	5									
Menard and Hurxthal (12)	115	Yes		?						T	115	1 to 24	"very little change in uncomplicated hyperthyroidism"											
Lerman and Means (14)	399	No		32				10		P														
Jones, Seabrook, Menne (15)	827	Yes		33				10		P														
Burnett and Durbin (16)	148	Yes		30						P+T														
Margolies, Rose, Wood	137	No		26				14		0	61T 25NT	12+ 12+	0 0	7 1	8 3	10 8	4 0	29 9	3 2	0 0	0 2	11	16	

\* Method used to determine heart size, P, percussion; T, teleroentgenograms; O, orthodiagrams.

Postmortem studies on thyrotoxic patients confirm the presence of cardiac hypertrophy in 50 to 75 per cent of cases (5, 11, 17). (Many of these patients, however, had associated cardiovascular disease.) Furthermore, thyroid feeding in dogs causes cardiac hypertrophy, which involves all chambers of the heart with slightly greater proportional increase in the left ventricle (18).

#### METHOD

We employed the orthodiagraphic method to record the contours of the heart. In our opinion this method, when carefully performed, is more reliable and accurate than percussion and teleroentgenography. The patients were examined in the erect posture, in the four standard posi-

tions.<sup>3</sup> The diaphragm and heart borders were drawn at the end of normal inspiration. Cardiac size was determined by measuring the frontal area, transverse diameter and the anteroposterior diameter. To obtain the frontal area it is necessary to complete arbitrarily the upper and lower borders of the heart by continuing the curves of the right and left borders toward the midline (Figure 1). No attempt was made to obtain the systolic and diastolic size of the heart for two reasons: (1) the difficulty of doing this in a rapidly beating heart, and (2) the difference in frontal area in the two phases of the heart beat

<sup>3</sup> Anteroposterior position, right anterior oblique position at 45 degrees, left anterior oblique position at 45 degrees and the left lateral position.

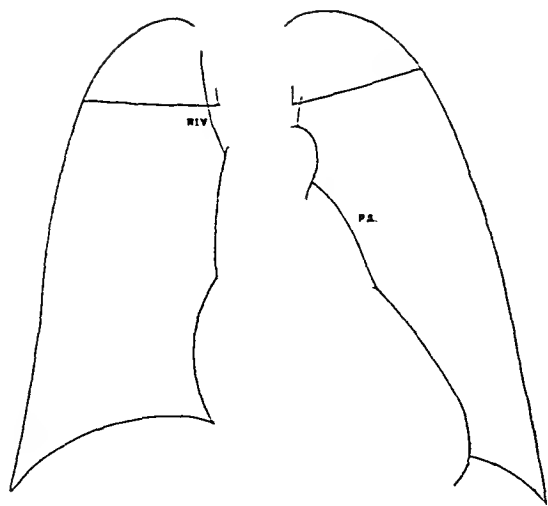


FIG. 1. TYPICAL ORTHODIAGRAM IN HYPERTHYROIDISM, SHOWING INCREASED PROMINENCE OF PULMONARY ARTERY (P. A.).

The broken line indicates the position of the border of the pulmonary artery after relief of hyperthyroidism. The curved broken lines show the method of arbitrarily completing the upper and lower borders of the heart to obtain the frontal area. (See text).

would fall within the limits of error of the method and within the range of normal variation.

#### *Relative heart size*

Simple empiric observation of the relative sizes of the heart and thoracic cage has been superseded to a considerable extent by more exact measurements (19). Diagonal measurements are unreliable, in our experience, because of their variability with cardiac position and their wide normal range. Determination of cardio-thoracic ratio in a series of normals has convinced us that the range of normal variation is too wide to be of value in the determination of cardiac enlargement in the individual case. The position of the heart in the chest and the cross-sectional shape of the chest are so variable normally that attempts to relate heart size to chest measurements are subject to error. This criticism applies also to attempts to measure differential enlargements of the ventricles by the median right and median left diameters. Many normal hearts occupy a central or a left lateral position without respect to a more or less transverse position. No reliable method has yet been devised to determine roentgeno-

graphically the relative contributions of the right and left ventricles in an enlarged heart (20). Alterations in shape may, however, suggest preponderance of one or the other ventricle. Determination of the size and shape of the heart shadow does not, of course, give any information as to whether increase in size is due to true hypertrophy, dilatation of the chambers, or to both.

It seems more reasonable to relate heart size to body size in the individual. Theoretically, the ideal would be the heart-volume, body-size ratio, but in the absence of criteria for that correlation we placed greatest reliance on the frontal area and transverse diameter in relation to height and weight, and made use of the tables of Hodges and Eyster (21, 22). The heart size was considered within normal limits when the area was not more than 15 per cent above or below the predicted normal for height and weight,<sup>1</sup> and the transverse diameter less than 1.5 cm. from the prediction. All graphs and tables, however, refer to area alone. The anteroposterior diameter of the heart was used to determine the normality of heart depth. We believe that if a sufficiently wide range of normal variation is allowed, cardiac area may be accepted as a reliable index of heart size in patients whose anteroposterior cardiac diameter falls within the average normal range (23). Two patients were excluded from our analysis because their hearts presented a definitely enlarged frontal area, but with anteroposterior diameters much below the average normal range due to compression in a shallow chest.

The heart size was considered to have changed significantly when the variation in area was 10 per cent or more of the measurement with which it was compared. This standard was adopted so as to include the maximum possible technical error and the change in predicted heart size due to change in weight.<sup>2</sup>

Our analysis of changes in the orthodiagram as shown in the accompanying figures has included only those patients without associated conditions

<sup>1</sup> Normal hearts are rarely found to be more than 15 per cent above the predicted area.

<sup>2</sup> From the tables of Hodges and Eyster (21, 22) it was computed that the cardiac area should vary 3 per cent for each 20 pounds change in weight, heights remaining the same.

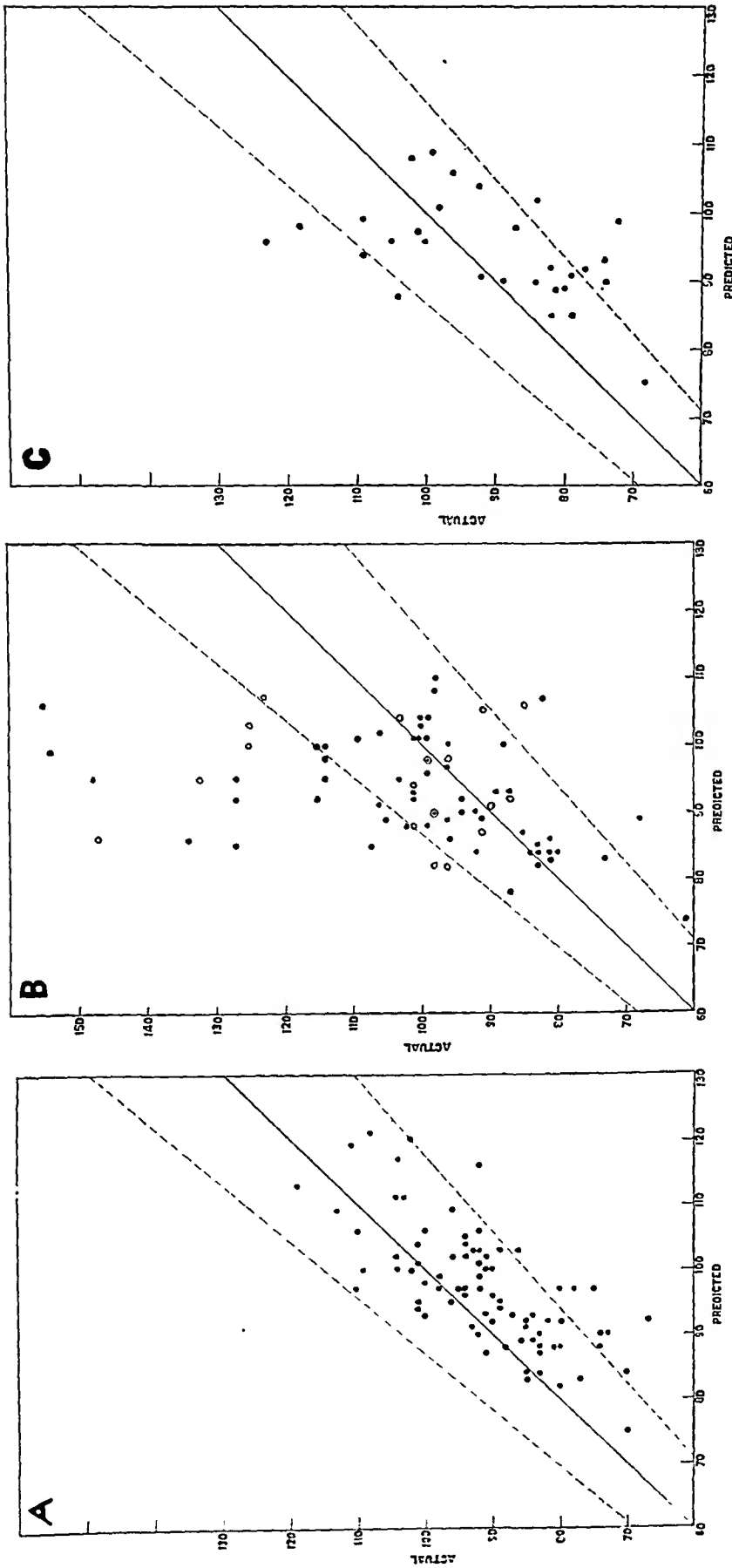


FIG. 2.

A. Relation between predicted and actual cardiac areas in a group of 60 females and 20 males without cardiovascular or thyroid disease. The abscissa represents predicted area, and the ordinate the actual area. (Measurements of cardiac area are expressed in all figures in sq. cm.) The area within the broken lines includes measurements 15 per cent above or below the predicted cardiac area; this we have arbitrarily set as the limit of normal variation. The same zone is indicated in Figures 2B, 2C, 6A, B and C.

B. Relation between predicted and actual cardiac areas in a group of 60 females and 20 males with thyrotoxicosis, before operation. Circles indicate patients incompletely relieved of thyrotoxicosis at the time of last examination. There are five coincidences, two of which are indicated by dots within circles; the other three are dot coincidences.

C. Relation between predicted and actual cardiac areas in 28 patients with non-toxic goiter, before operation.

(particularly cardiovascular lesions) which might affect the size and shape of the heart. This has resulted in the exclusion of 21 of the toxic group and 7 of the non-toxic group. Two patients with persistent auricular fibrillation, but without signs of failure, were included (Figure 5, A. F.).

Figure 2*A* shows the relation between the actual and predicted cardiac areas in a group of 60 females and 20 males without cardiovascular or thyroid disease. The ages of the females ranged from 14 to 61 years, with an average of 34.8 years; the ages of the males ranged from 14 to 60 years, with an average of 38.9 years.

Figure 2*B* shows the same relation between actual and predicted area in a group of 60 females and 20 males with thyrotoxicosis. The females varied in age from 14 to 61 years, with an average of 36.7 years; the males varied in age from 13 to 59, with an average of 35.6 years. Twenty-six per cent of the thyrotoxic group had enlarged hearts, and 5 per cent had small hearts. In the control group (Figure 2*A*) cardiac enlargement was present in none, and 12.5 per cent had small hearts.

Fourteen per cent of 28 patients with non-toxic goiter showed enlarged hearts, and 18 per cent small hearts (Figure 2*C*). Twenty-seven of this group were females, ranging in age from 14 to 61 years, with an average age of 39. The one male was 44 years old.

Figure 3 shows individual variations in cardiac area throughout the period of observation in the 28 patients with non-toxic goiters. Twenty-five of these patients were followed for one year or more after operation. Examination of this figure shows that cardiac enlargement was present before operation in 4 cases (14 per cent); 3 of these showed no significant change in size after operation; one became smaller. Increase in cardiac area after operation occurred in 11 (39 per cent); 8 were normal in size and 3 were small before operation. Sixteen (57 per cent) showed no significant ultimate change. Of the 5 hearts classified as small before operation, 3 increased in size postoperatively and 2 were unchanged.

Figures 4 and 5 represent similar individual variations in 80 thyrotoxic patients (one case is not recorded because of insufficient follow-up data). Sixty-one of these patients were followed

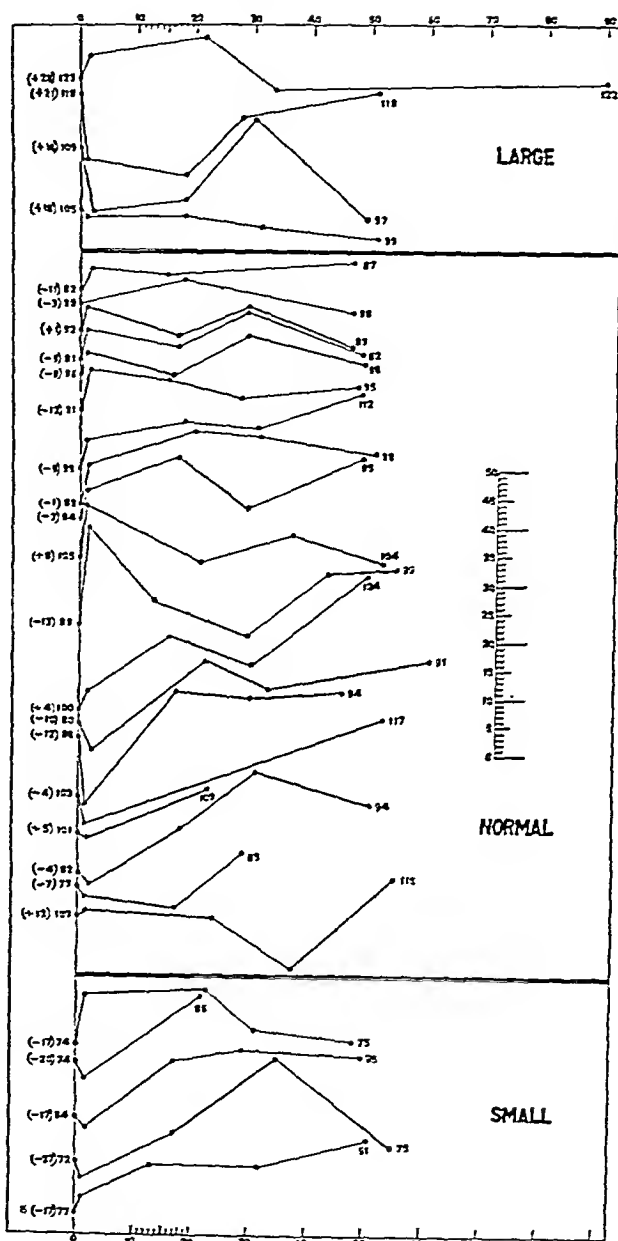


FIG. 3. INDIVIDUAL VARIATIONS IN CARDIAC AREA THROUGHOUT THE PERIOD OF OBSERVATION IN 28 PATIENTS WITH NON-TOXIC GOITER.

Thyroidectomy was done in all cases between the first and second measurements. Abscissa represents weeks of observation. The percentage relation to predicted area is expressed by the figures in parentheses in the column on the left; other figures represent actual cardiac area before operation and at the last examination after operation. G = patient growing throughout the period of observation. The scale (in sq. cm.) may be used to determine variations in area between any two measurements on the same curve. Cases in Figures 3, 4 and 5 are grouped according to heart size before operation.

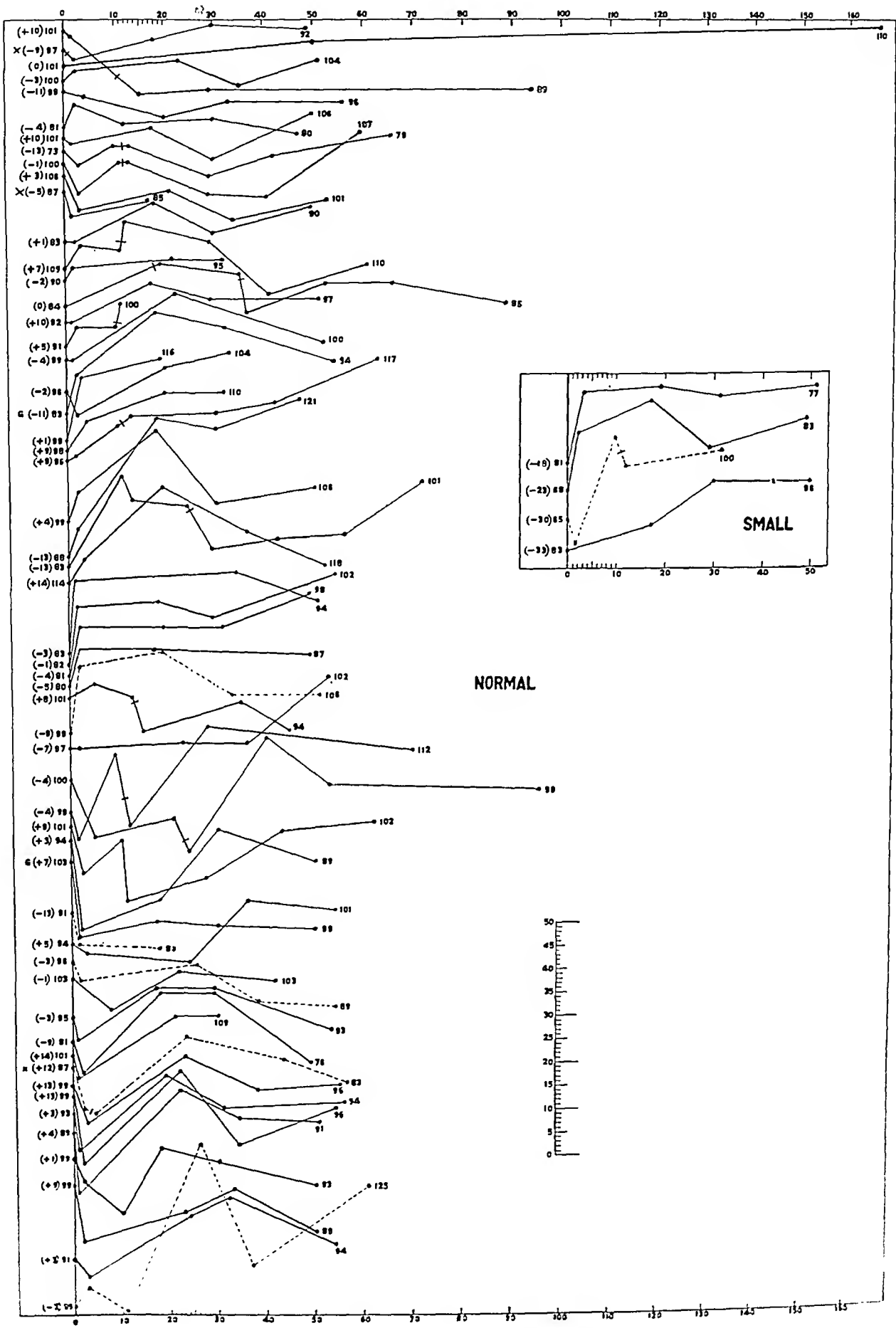


FIG. 4.

See Figure 5 for legend.

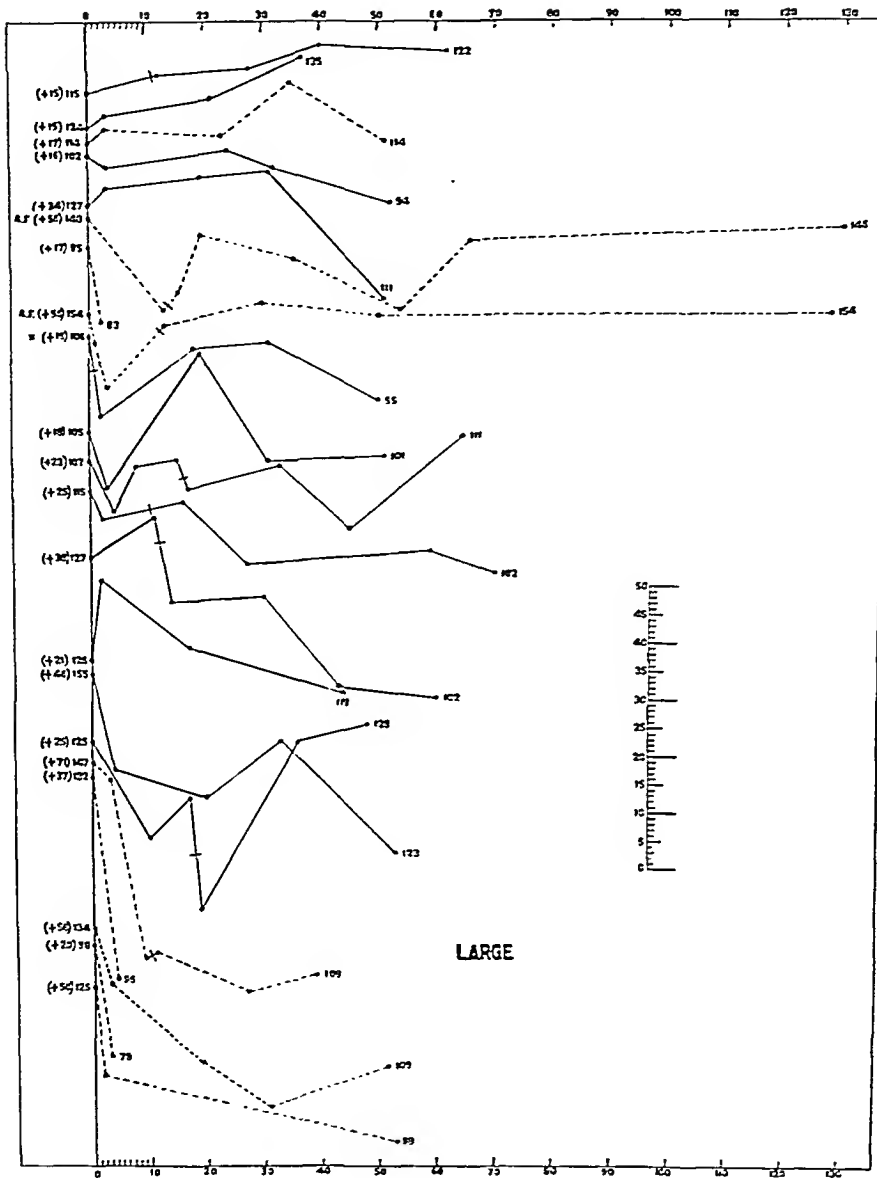


FIG. 5.

FIGS. 4 AND 5. INDIVIDUAL VARIATIONS IN CARDIAC AREA THROUGHOUT THE PERIOD OF OBSERVATION IN 80 PATIENTS WITH THYROTOXICOSIS.

Thyroidectomy was done between the first and second measurements in all cases except those marked with an x (4 cases), in which operation was done before the first measurements. The short perpendicular cross lines indicate the time of a second operation, usually a hemi-thyroidectomy. The eleventh curve from the top (Figure 4) is that of a patient who had only one operation, and in whom cardiac measurements were first recorded 3 days postoperatively. The cases incompletely relieved of thyrotoxicosis are indicated by broken lines. AF=auricular fibrillation. G=patient growing during observation.



for one year or more after operation: of these, 52 were completely relieved of their thyrotoxicosis at the time of the last examination, and 9 showed evidence of incomplete relief. Preoperative cardiac enlargement was almost twice as frequent (21 cases or 26 per cent) as in the non-toxic group. Significant ultimate changes in size occurred in 31 (39 per cent). None of the enlarged hearts showed further increase in size, and 11 showed reduction, including 6 in patients who were incompletely relieved of their thyrotoxicosis. All of the 4 small hearts became larger after operation. Of the 55 patients with hearts of normal size before operation, 12 increased significantly in size after operation, 39 remained unchanged, and 4 became smaller. Cases presented in Figures 3, 4 and 5 which were followed for one year or more are included in Table I.

The frequency with which cardiac enlargement occurred in our cases of uncomplicated thyrotoxicosis seems to be significant. The number of cases of non-toxic goiter studied was not large enough to warrant any definite conclusions regarding the incidence of cardiac enlargement, but its frequency was greater than normal. The average degree of enlargement (expressed in terms of percentage increase above the predicted area) in the toxic group (21 cases) was 32 per cent, and in the non-toxic group (4 cases) 21 per cent. Of the 21 thyrotoxic patients with enlarged hearts, 19 had diffuse goiters, and 2 nodular goiters.

Of 53 patients in the thyrotoxic group, eleven per cent showed enlargement one year or more after successful thyroidectomy; the remainder were within the normal zone (Figure 6A; cf. Figure 2B).

Of 12 thyrotoxic patients who were incom-

pletely relieved, nine were followed for one year or more, and one each for 20, 30 and 40 weeks respectively. Six showed change in actual area of less than 10 per cent, 4 had decreased 10 per cent or more, and only 2 showed increase in size (Figure 6B).

Figure 6C shows the relation between actual and predicted areas in 25 patients with non-toxic goiter one year or more after operation. Comparison of the relative proportion of large, small and normal hearts in 25 patients with non-toxic goiter before and one year after operation shows no significant shift (Figure 2C, cf. Figure 6C).

We were unable to demonstrate any significant relation between the duration of thyrotoxicosis or of non-toxic goiter, as determined roughly from the history, and the incidence of cardiac enlargement. In the thyrotoxic patients without heart failure some of the most marked cardiac enlargements occurred in cases of apparently short duration. Because of the marked tendency to spontaneous fluctuation in the intensity of thyrotoxicosis, we made no attempt to correlate this factor with abnormalities of heart size.

The relation between changes in body weight and cardiac area during the period of observation is shown in Table II. Without significant weight change, the non-toxic patients showed a greater tendency to significant increase in heart size than did the toxic group with cardiac compensation preserved. The latter showed a more marked tendency to gain weight after operation, as might have been anticipated. Actual variations in cardiac area bore no direct relation to changes in predicted area based on weight changes. There did, however, seem to be a tendency toward increase in heart size with increasing gain of weight (compare columns A in Table II).

TABLE II  
*The relation between changes in body weight and cardiac area*

Ultimate change in weight, per cent. . . . .	- 10 to + 10			+ 10 to + 20			+ 20 to + 30			+ 30 to + 40			+ 40 to + 50			> +65
Ultimate change in area*	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A
Toxic . . . . .	2	20	5	5	24	0	3	8	4	3	0	0	1	0	1	1
Non-toxic . . . . .	10	14	0	2	1	1	0	0	0	0	0	0	0	0	0	0

\* A—Ultimate increase in area of 10 per cent or more.  
B—Ultimate change in area of less than 10 per cent.  
C—Ultimate decrease in area of 10 per cent or more.

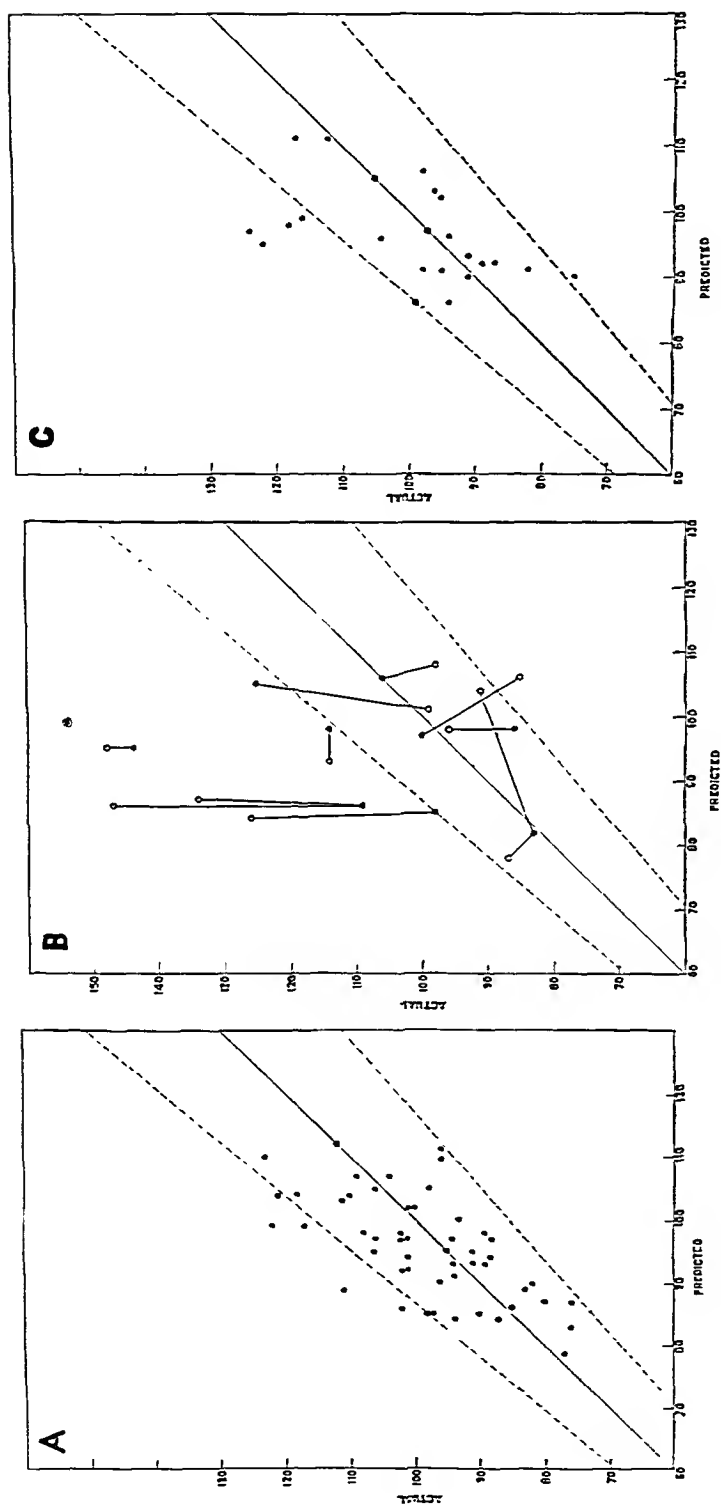


FIG. 6.

- A. Relation between predicted and actual cardiac areas in 53 patients one year or more after operative relief of thyrotoxicosis.  
 B. Relation between predicted and actual cardiac areas before and at the last observation after thyroidectomy in 12 thyrotoxic patients incompletely relieved by operation. Circles indicate preoperative relation.  
 C. Relation between predicted and actual cardiac areas in 25 patients one year or more after operation for non-toxic goiter. (One coincidence.)

Nine toxic patients showed either a reduction of the basal metabolic rate to —15 per cent or below, or exhibited other evidence of hypothyroidism after operation. In 5 these changes were not associated with any significant variation in cardiac area. In 4 there was an increase in heart size varying from 9 to 18 per cent up to the time of appearance of the thyreoprival signs. In 3 of these patients there was coincidental weight gain. In 3 non-toxic patients the basal metabolic rate dropped after operation to —15 per cent or below, and in 2 others mild thyreoprival symptoms appeared. In the latter two no change in cardiac area occurred. Of the former, 2 showed increase in cardiac area of 11 and 12 per cent respectively without significant weight gain. It appears that postoperative hypothyroidism may be partly responsible for increase in cardiac area occurring after thyroidectomy (13).

Sex did not seem to influence the incidence of cardiac enlargement in the toxic group. All but one of the non-toxic patients were females.

Table III shows the age distribution with reference to the incidence of cardiac enlargement. The

TABLE III

*The relation between age and the incidence of cardiac enlargement (15 per cent or more above prediction)*

Decade	Toxic		Non-toxic	
	Total number of cases	Number with large hearts	Total number of cases	Number with large hearts
2.....	5	0	3	0
3.....	18	4	3	1
4.....	23	6	11	1
5.....	25	8	7	0
6.....	8	5	3	1
7.....	1	0	1	1

frequency of enlargement appears to increase directly with age in the toxic patients, but there is no apparent relationship in the non-toxic group.

Fifteen (19 per cent) of 81 toxic patients showed temporary variations of 10 per cent or more in cardiac area during the period of observation. In these cases there were no significant ultimate changes in area from the first to the last measurements. These variations did not bear any relation to changes in heart rate or blood pressure. It is not certain that thyrotoxicosis influ-

enced these variations in size as no adequate control observations have been made in normals.

Eight toxic patients with preserved compensation received digitalis before or after operation in moderate dosage for short periods without apparent effect on their cardiac measurements.

Nine thyrotoxic patients and one with non-toxic goiter, not included in the foregoing discussion, were in congestive heart failure when first seen. All showed increase in cardiac area, varying from 9 to 62 per cent above prediction. Their individual variations in heart size are shown in Figure 7. Seven of the nine thyrotoxic patients were

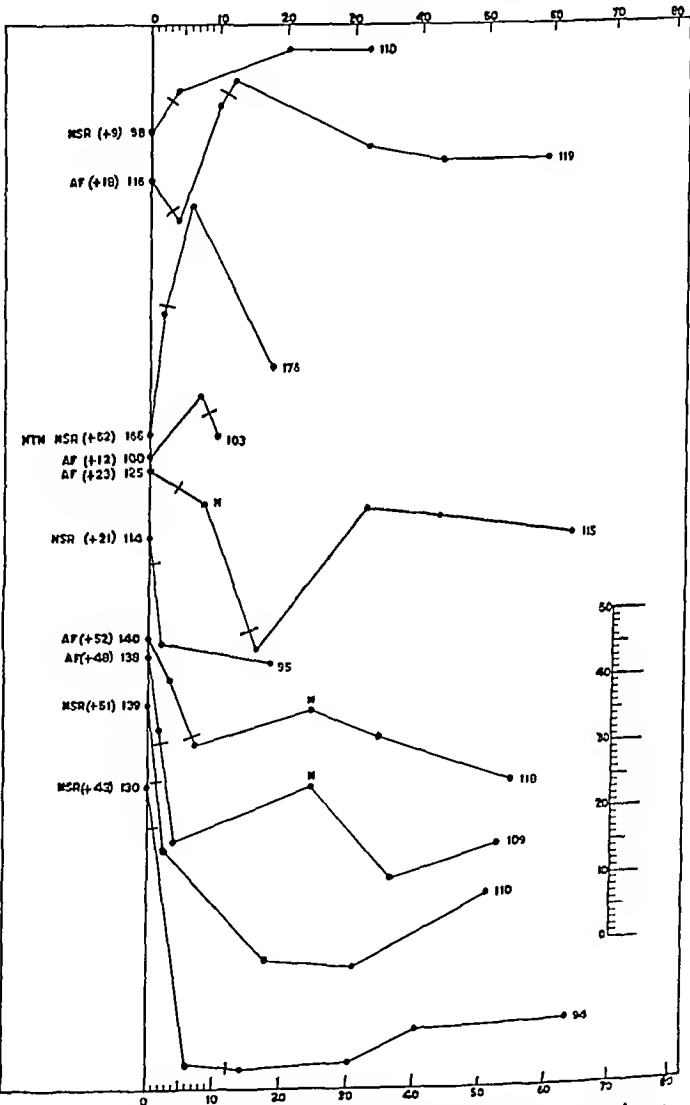


FIG. 7. INDIVIDUAL VARIATIONS IN CARDIAC AREA THROUGHOUT THE PERIOD OF OBSERVATION IN 10 PATIENTS WITH CONGESTIVE HEART FAILURE BEFORE OPERATION; ALL WERE THYROTOXIC EXCEPT ONE (NTN) WHO HAD A NON-TOXIC, NODULAR GOITER WITH HYPERTENSION AND CARDIOVASCULAR AND RENAL DISEASE.  
NSR=normal sinus rhythm. AF=auricular fibrillation. N=the first measurement after re-establishment of normal sinus rhythm.

relieved of their thyrotoxicosis after operation. Five were in auricular fibrillation; in 3 normal rhythm returned postoperatively. In 6 the cardiac area diminished after operation, despite gain of weight in 5. One patient was followed for only a few days after operation, and her fate is unknown. The one patient known not to have been completely relieved of thyrotoxicosis showed a slight increase in cardiac area 11 months after operation. Eight of the nine toxic patients were relieved of their decompensation (including the one who remained thyrotoxic). The one non-toxic patient (a male) had cardiovascular-renal disease with hypertension and heart failure; he was unimproved four and a half months after operation.

### *Tracheal stenosis*

The consensus in the literature is that tracheal stenosis from thyroid pressure has no demonstrable effect on the size of the heart. Parkinson and Cookson (11), in postmortem examinations of 43 thyrotoxic patients, found cardiac hypertrophy in 22, but only 2 of these showed tracheal stenosis. We made no systematic attempt to analyze the incidence or degree of severity of tracheal stenosis. We have, however, observed a number of patients with substernal goiter causing pressure symptoms, in whom there was no cardiac enlargement.

### *Pulmonary artery*

Bauer and Helm (24) first called attention, in patients with thyroid disease, to the prominence and increased pulsation of the pulmonary artery and pointed out that increased prominence and increased pulsation are not always combined. Parkinson and Cookson (11) found a definite prominence of this arc in 42 (32 per cent) of 130 thyrotoxic patients. The heart was normal in size in over one third (16) of the cases with definite prominence. They confirmed the presence of dilatation of the pulmonary artery by postmortem examination and pointed out that it may be obscured during life by the position of the heart. They attributed the dilatation to the widened pulse pressure and increased heart rate in thyrotoxicosis. Menard and Hurxthal (12) found a prominent (straight or convex) pulmonary arc in 52 (45 per cent) of their 115 toxic patients. They further

report the same finding in 30 per cent of their patients with non-toxic goiter and in 32 per cent of non-goitrous individuals but think the prominence is greater in thyrotoxicosis. They observed a definite reduction after thyroidectomy or after disappearance of heart failure in 19 (37 per cent) of their toxic patients.

In our series of 81 thyrotoxic patients, 44 (54 per cent) showed prominence and/or increased pulsation of the pulmonary artery (Figure 1). Of these, 35 showed prominence and increased pulsation combined. Four showed prominence alone, and 5 increased pulsation alone. Of the 28 patients with non-toxic goiter, 4 (14 per cent) showed one or the other of these pulmonary artery changes. This group included one patient with slight prominence of the artery, in whom a diagnosis of chronic thyroiditis was made. Another patient showed slight dilatation of the pulmonary artery in the right anterior oblique position but not anteroposteriorly. Two patients showed increased pulsation without prominence of the artery.

In 34 of the 44 toxic patients, the abnormality of the pulmonary artery disappeared in from 1 to 7 months after operation (average 2.3 months). In this group, 16 hearts became smaller, 12 became larger, and 6 did not change in size. In the 10 patients in whom the changes persisted, 8 were classed as successful results, one was unsuccessful, and one was not followed long enough to be classified. Of these 9 patients followed, the cardiac area decreased in 2 and was unchanged in 7. In 3 of the 4 non-toxic patients, the changes in the pulmonary artery disappeared in 1, 4 and 12 months respectively without significant change in heart size. In the fourth case there was no change one year after thyroidectomy.

Seven patients with other complicating conditions showed abnormalities of the pulmonary artery before operation. Three were in decompensation; the others had aortic insufficiency, mitral insufficiency, mitral stenosis and hypertension, respectively. The abnormality disappeared after operation in all but the two patients with mitral lesions.

We were unable to demonstrate any relationship between the occurrence of changes in the pulmonary artery and (a) changes in heart size, (b) systemic blood pressure, (c) preoperative heart

Nine toxic patients showed either a reduction of the basal metabolic rate to —15 per cent or below, or exhibited other evidence of hypothyroidism after operation. In 5 these changes were not associated with any significant variation in cardiac area. In 4 there was an increase in heart size varying from 9 to 18 per cent up to the time of appearance of the thyreoprival signs. In 3 of these patients there was coincidental weight gain. In 3 non-toxic patients the basal metabolic rate dropped after operation to —15 per cent or below, and in 2 others mild thyreoprival symptoms appeared. In the latter two no change in cardiac area occurred. Of the former, 2 showed increase in cardiac area of 11 and 12 per cent respectively without significant weight gain. It appears that postoperative hypothyroidism may be partly responsible for increase in cardiac area occurring after thyroidectomy (13).

Sex did not seem to influence the incidence of cardiac enlargement in the toxic group. All but one of the non-toxic patients were females.

Table III shows the age distribution with reference to the incidence of cardiac enlargement. The

TABLE III

*The relation between age and the incidence of cardiac enlargement (15 per cent or more above prediction)*

Decade	Toxic		Non-toxic	
	Total number of cases	Number with large hearts	Total number of cases	Number with large hearts
2.....	5	0	3	0
3.....	18	4	3	1
4.....	23	6	11	1
5.....	25	8	7	0
6.....	8	5	3	1
7.....	1	0	1	1

frequency of enlargement appears to increase directly with age in the toxic patients, but there is no apparent relationship in the non-toxic group.

Fifteen (19 per cent) of 81 toxic patients showed temporary variations of 10 per cent or more in cardiac area during the period of observation. In these cases there were no significant ultimate changes in area from the first to the last measurements. These variations did not bear any relation to changes in heart rate or blood pressure. It is not certain that thyrotoxicosis influ-

enced these variations in size as no adequate control observations have been made in normals.

Eight toxic patients with preserved compensation received digitalis before or after operation in moderate dosage for short periods without apparent effect on their cardiac measurements.

Nine thyrotoxic patients and one with non-toxic goiter, not included in the foregoing discussion, were in congestive heart failure when first seen. All showed increase in cardiac area, varying from 9 to 62 per cent above prediction. Their individual variations in heart size are shown in Figure 7. Seven of the nine thyrotoxic patients were

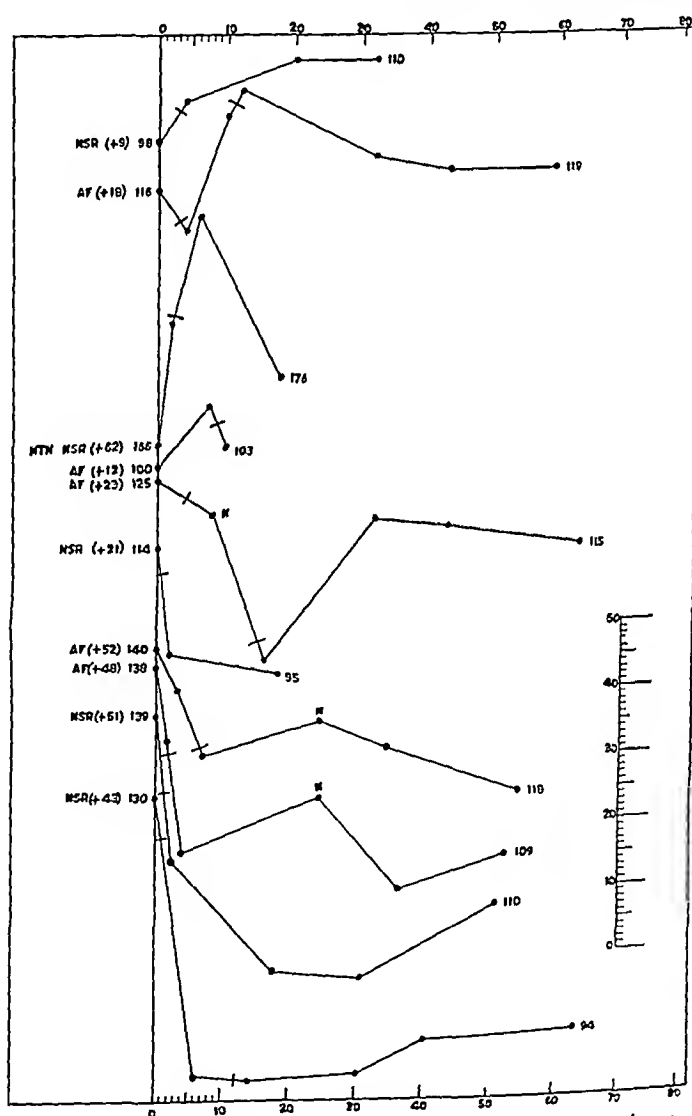


FIG. 7. INDIVIDUAL VARIATIONS IN CARDIAC AREA THROUGHOUT THE PERIOD OF OBSERVATION IN 10 PATIENTS WITH CONGESTIVE HEART FAILURE BEFORE OPERATION; ALL WERE THYROTOXIC EXCEPT ONE (NTN) WHO HAD A NON-TOXIC, NODULAR GOITER WITH HYPERTENSION AND CARDIOVASCULAR AND RENAL DISEASE.

NSR = normal sinus rhythm. AF = auricular fibrillation. N = the first measurement after re-establishment of normal sinus rhythm.

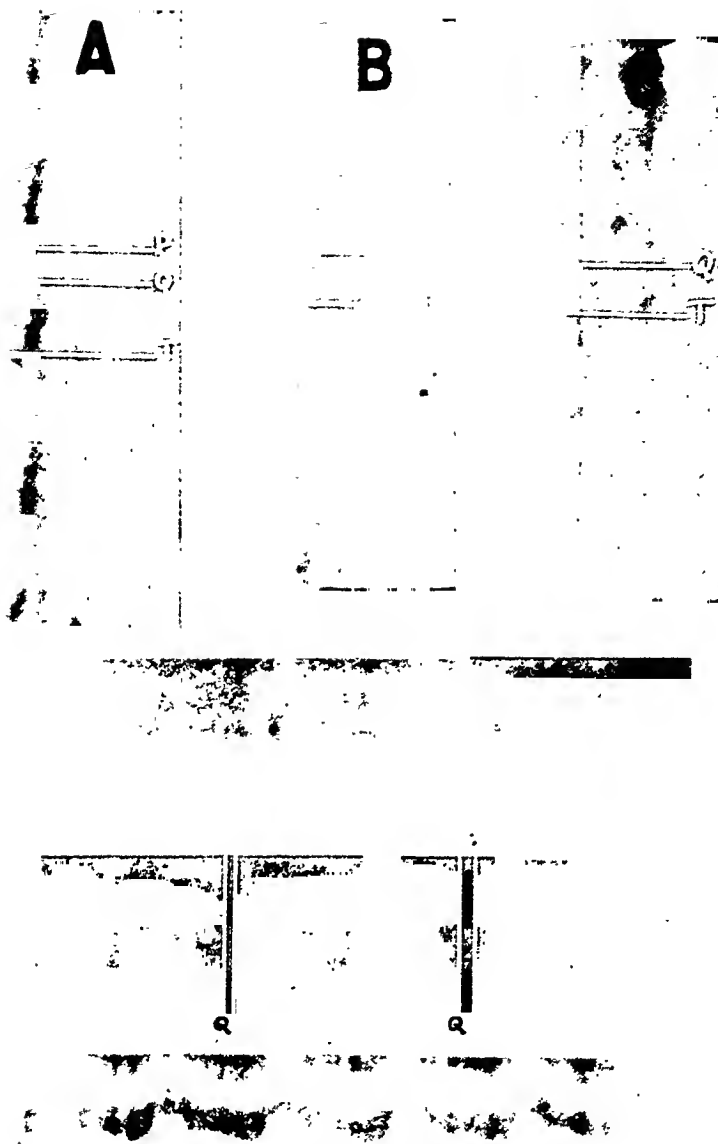


FIG. 8. ROENTGEN-KYMOGRAMS OF THE BORDERS OF THE LEFT VENTRICLE AND PULMONARY ARTERY.

The lung fields show as the lighter zones; the heart and pulmonary artery respectively produce the black zones; the serrated edges represent the cardiac and vascular pulsations. The ventricular tracings (*A*, *B* and *C*) should be read from above downward; systole is represented by the deflection of the heart border to the left, diastole by the deflection to the right. The arterial tracings (*D*, *E* and *F*) should be read from left to right; systole is represented by the upward deflection of the vascular shadow, as in the usual pulse tracing. The letter *P* identifies the position of the beginning of the *P* wave in the simultaneously recorded electrocardiogram; *Q*, the beginning of the *QRS* complex; *T*, the end of the *T* wave (27, 28).

*A*. Pulsation of the left ventricular border in a normal heart, rate 110.

*B*. Pulsation of the left ventricular border in a normal heart, rate 140.

*C*. Pulsation of the left ventricular border in a thyrotoxic heart, rate 136. The systolic contraction is quicker and greater in extent than in the normal heart beating at approximately the same rate.

*D*. Pulsation of the pulmonary artery in a normal patient, heart rate 115.

*E*. Pulsation of the pulmonary artery in a thyrotoxic patient, heart rate 150 (faster moving cassette).

*F*. Pulsation of the pulmonary artery in a thyrotoxic patient, heart rate 110. The amplitude of the arterial pulsation is greater in the thyrotoxic patients.

rate, or (d) basal metabolism, in either the toxic or non-toxic group. The relative importance of alterations in pressure within the pulmonary circulation in this connection cannot be determined at present.

### *Cardiac pulsation*

Kraus (1) first called attention to the marked change in heart size during contraction in hyperthyroidism. Bauer and Helm (24) noted the increased pulsation of the whole left border. Rösler (25) believes that the pulsation is characteristic in that the systolic contraction is quicker than in a normal heart beating at the same rate; that the contraction is not wave-like as in normals, but the whole ventricle seems to contract at the same time. Our own fluoroscopic observations supported by roentgen-kymographic studies (27, 28) of the pulmonary artery and left ventricular pulsations (Figure 8), confirm the presence of the characteristic pulsation in hyperthyroidism. This characteristic pulsation disappears after relief of hyperthyroidism.

### *Cardiac shape*

The following characteristics of the cardiac configuration in hyperthyroidism have been described: prominence of the middle left border (pulmonary artery) (9, 11, 24, 25, 26); prominence and increased width of the superior vena cava (11, 25); high aortic knob (24); rounded apex (24); a mitral configuration (9); a ham-like contour (11); cardiac shape and pulsation may, at times, first draw the attention of the clinician to the existence of thyrotoxicosis (9, 25). The lung fields may be unusually clear (25, 26).

We found that, at times, the anteroposterior cardiac silhouette partially simulates the "mitral configuration," because of the filling out of the normally concave middle left border by the dilated pulmonary artery; the clouded lung fields and posterior enlargement of the left auricle seen in mitral stenosis are not present in hyperthyroidism. Furthermore, the aortic knob and its pulsation are not usually as obscure as in the case of mitral stenosis. The height of the aortic knob was not significantly different from that seen in a group of non-goitrous patients of corresponding age distribution (25). We did not find increased width of the superior vena cava (12) (middle

right border), but the extreme upper right vascular border was frequently somewhat oblique, probably due to right lateral displacement of the right innominate vein by the enlarged thyroid gland. We found nothing characteristic in the shape of the heart itself, exclusive of the vascular pedicle. When the heart was enlarged, the configuration suggested bilateral increase with probably slight left ventricular preponderance in most cases.

### SUMMARY

Orthodiagraphic studies have been made in 102 thyrotoxic patients and 35 patients with non-toxic goiter. These studies were made before partial or subtotal thyroidectomy and at successive intervals thereafter up to one year in most instances. Our findings have been analyzed with respect to the following: (1) the incidence of abnormalities of cardiac area before operation; (2) changes in cardiac area following complete and incomplete surgical relief; (3) the relation between changes in cardiac area and (a) duration of goiter or thyrotoxicosis, (b) postoperative weight changes, (c) postoperative hypothyroidism, (d) age, (e) sex, (f) heart failure; (4) the incidence of changes in the appearance of the pulmonary artery, and the effect of thyroidectomy on these changes; and (5) the shape and character of pulsation of the heart in hyperthyroidism.

### CONCLUSIONS

1. Significant increase in cardiac area occurs frequently in uncomplicated hyperthyroidism (26 per cent of our cases). The incidence of such increase in patients with uncomplicated non-toxic goiter is also probably abnormal (14 per cent in our series of 28 cases).

2. Following thyroidectomy in uncomplicated hyperthyroidism, there is a tendency for hearts of abnormal size, whether large or small, to return toward normal and for hearts within the normal zone to remain so. This tendency is not materially affected by failure to control completely the hyperthyroidism.

3. Significant changes in cardiac area do not occur following thyroidectomy for uncomplicated non-toxic goiter.

4. Postoperative hypothyroidism may occasionally be a factor in increasing cardiac area.

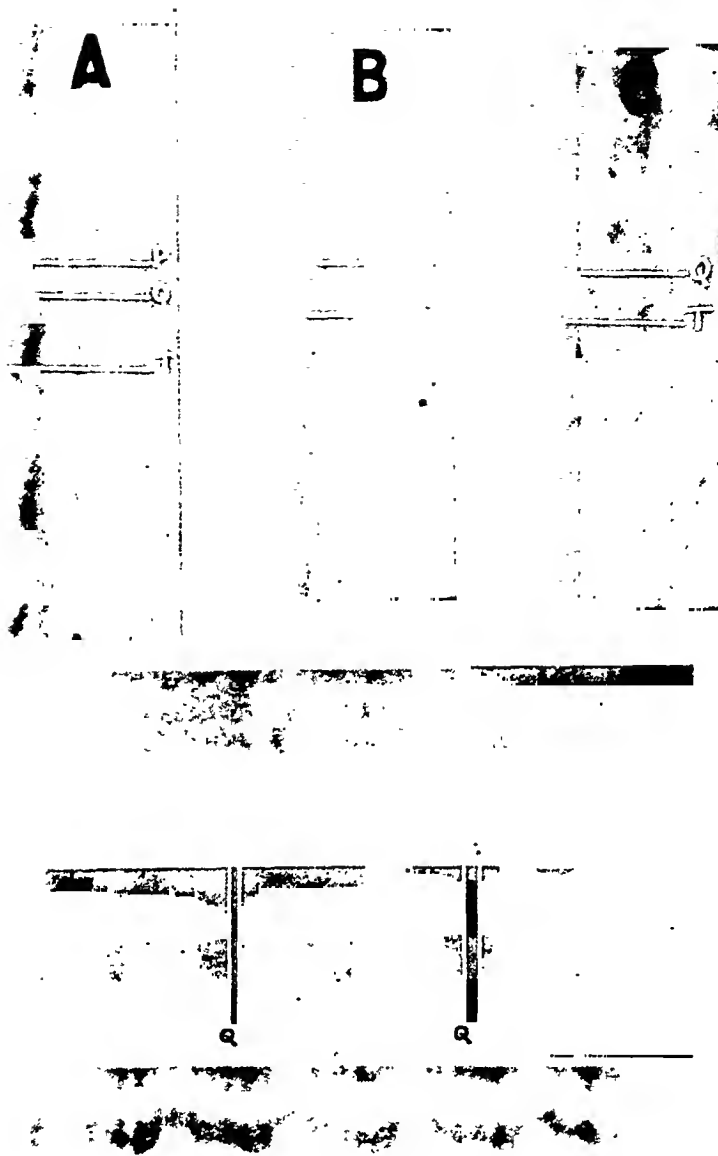


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- D. Pulsation of the pulmonary artery in a normal patient, heart rate 115.
- E. Pulsation of the pulmonary artery in a thyrotoxic patient, heart rate 150 (faster moving cassette).
- F. Pulsation of the pulmonary artery in a thyrotoxic patient, heart rate 110. The amplitude of the arterial pulsation is greater in the thyrotoxic patients.



5. Temporary variations in cardiac area, without significant ultimate changes, occur after thyroidectomy for uncomplicated hyperthyroidism in some cases (19 per cent in our series). The cause of these variations is not known.

6. Congestive heart failure in hyperthyroidism is almost always accompanied by enlargement of the cardiac area. This tends to decrease with postoperative restoration of compensation, provided the thyrotoxicosis is also relieved.

7. Increased prominence and/or pulsation of the pulmonary artery is frequent in hyperthyroidism (54 per cent of our cases). It occurs less frequently in association with non-toxic goiter (14 per cent of our cases). The cause of these changes is unknown. We were unable to demonstrate any relation between them and (a) changes in heart size, (b) systemic blood pressure, (c) preoperative heart rate, or (d) basal metabolism, in patients with toxic or non-toxic goiter. In about three-fourths of our cases, the abnormality of the pulmonary artery disappeared within 1 to 7 months after thyroidectomy.

8. Cardiac pulsation, as observed fluoroscopically, is usually characteristically altered in hyperthyroidism.

9. The heart (exclusive of the vascular pedicle) does not assume a characteristic shape in persons with toxic or non-toxic goiter.

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# THE HEART IN THYROID DISEASE. II. THE EFFECT OF THYROIDECTOMY ON THE ELECTROCARDIOGRAM<sup>1</sup>

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(Received for publication March 28, 1935)

The purpose of these observations has been to determine (1) what abnormalities, if any, occur in the electrocardiograms of persons with either toxic or non-toxic goiter; and (2) the electrocardiographic effect of partial or subtotal thyroidectomy in the same subjects. The plan of study has been described in a previous communication (1).

This report is based on a study of preoperative and postoperative electrocardiograms in 106 patients: 27 with non-toxic goiter, 20 with toxic nodular goiter, and 59 with toxic diffuse goiter. The records of 31 other patients were excluded because the electrocardiographic data were incomplete or because digitalis had been administered when one or more of the electrocardiograms were made.

The electrocardiograms were accurately standardized (1 cm. = 1 millivolt). Overshooting was avoided. All tracings were taken with immersion electrodes with the patient sitting. Comparison of skin resistances at the times of the first and last examinations in each instance showed no marked variations and no constant direction of change. Normal tracings were differentiated from abnormal ones by the usual criteria. Any T wave in Lead I or II, one millimeter or less in height, and any QRS complex showing definite left axis deviation, were considered abnormal. Slight tremor of the string occurred in about half the thyrotoxic patients at the first examination, and in a few instances this was marked; it usually disappeared later in the course of observation. All the thyrotoxic patients were receiving iodine when the preoperative tracing was taken, and this may have tended to reduce the height of the T waves (2). In determining whether or not the electrocardiogram of any given patient had changed, alterations of less than 1 millimeter in

T waves, and of less than 5 millimeters in QRS complexes, were not considered significant.

The electrocardiographic changes in hyperthyroidism have been considered at length in the literature. The most important contributions are those of Krumbhaar (3); Hamburger, Lev, Priest, and Howard (2); Jonás and Wichterlová (4); Coelho (5); Goodall and Rogers (6); Gossels (7); Willius, Boothby, and Wilson (8); Smith and Colvin (9); White and Aub (10); and Don and Langley (11). Don and Langley report studies similar to those described here. Some writers (3, 12, 13) believe that the electrocardiogram in hyperthyroidism is usually characteristic with large P and T waves. Others (9, 10, 14) disagree with this view and state that the T waves may be large, small or inverted and bear no relation to the degree of toxicity. Similar disagreement is found with regard to the effects of treatment. Thus Hamburger and his co-workers (2) state that relief of hyperthyroidism is usually accompanied by reduction in the height of the T waves, while Don and Langley (11) could find no relation between reduction of the basal metabolic rate and changes in P and T waves.

Table I contains a classification of the electrocardiograms in patients with various types of goiter and the changes which occurred in them after thyroidectomy.

*P waves.* Twenty-five of the 79 toxic cases had P waves 3 mm. or more in height in one or more leads at the preoperative examination. All but 4 of these showed a reduction to less than 3 mm. at the last examination. Two of these 4 were incompletely relieved of their thyrotoxicosis: the third had hypertension; and the fourth, mitral stenosis. In no instance did normal P waves in a preoperative tracing increase to abnormal height at the last examination. One patient in the non-toxic group had a P wave 3 mm. high before operation, which was reduced to 2 mm. at the last examination. The T waves in this case were of

<sup>1</sup> Presented in abstract before the Section on General Medicine, College of Physicians of Philadelphia, April 23, 1934.

TABLE I

*Classification of electrocardiographic abnormalities in various types of goiter*

Types of goiter.....	Non-toxic					Toxic nodular					Toxic diffuse					
	A*	B*	C*	D*	Total	A	B	C	D	Total	A	B	C	D	Total	Total toxic
Number of patients.....	16	0	4	7	27	11	2	2	5	20	33	11	9	6	59	79
P wave 3 mm. or higher.....				1	1	5				5	8	4	6	2	20	25
QRS complexes increased in size immediately after operation.....					0					0	3	1			4	4
All QRS complexes smaller at last examination than at first.....	1				1					0	6	3	1	1	11	11
Shift of electrical axis toward the left from first to last examination.....	1		1	2	4	6	1	1	1	9	18	7	5	1	31	40
T waves 4 mm. or higher at first examination.....	3			3	6	4	1		1	6	8	4		1	13	19
T waves increased in size immediately after operation..			1		1			1	1	2	8	3	5	2	18	20
Slight temporary reduction of T waves after operation..	5		1	1	7	4		1	1	6	4	3		2	9	15
Temporary reduction of T waves to abnormal degree after operation.....	3				3	2				2	6	2	2	1	11	13
T waves smaller at last examination than at first.....	1			1	2	4	2			6	6	5			11	17
T waves larger at last examination than at first.....	2		4		6	3		2		5	4		4	1	9	14
T waves 4 mm. or higher at last examination.....	3†			3‡	6	3§			1	4	6	2¶	2		10	14

\* A—Normal at first and last examination.

B—Normal at first and abnormal at last examination.

C—Abnormal at first and normal at last examination.

D—Abnormal at first and abnormal at last examination.

† Present at first examination in 1 case.

‡ Present at first examination in all cases.

§ Present at first examination in 2 cases.

|| Present at first examination in 4 cases.

¶ Present at first examination in 1 case.

very low amplitude throughout, but there was no other evidence of cardiovascular disease, and nothing to indicate thyrotoxicosis. One thyrotoxic patient, whose other tracings were normal, showed inverted P waves in all leads at her last examination.

Auricular flutter was present before operation in one thyrotoxic patient; this was followed immediately after operation by auricular fibrillation. Normal rhythm was subsequently established. Auricular fibrillation was present in 12 other cases of hyperthyroidism. In 2 it was a temporary postoperative phenomenon. In 10 it was present at the first examination: in 7 of these it disappeared soon after operation, and in the other 3 it has persisted.

*P-R interval.* A minor grade of heart block was present throughout the period of observation in one non-toxic patient ( $P-R$  interval = 0.24 second). No other instance of disturbed auriculo-ventricular conduction was observed.

*QRS complexes.* Four toxic patients showed a temporary postoperative increase in the size of their QRS complexes. This was not seen in any

of the non-toxic group. In 11 of the toxic patients, the QRS complexes were definitely smaller in all leads at the last examination than at the first. This occurred in one of the non-toxic group. One toxic patient showed an excessively high complex before operation, which was later reduced to normal. In another instance left axis deviation appeared six months after operation but had disappeared one year after operation. One thyrotoxic woman, aged 29, showed a right bundle branch defect (new nomenclature) throughout the period of observation (3 years) and has shown no other evidence of cardiovascular disease.

The variations of the QRS complex described above were relatively infrequent, and their significance is difficult to determine. However, a definite shift of the electrical axis toward the left from the first to the last examination was noted in 40 (51 per cent) of the 79 toxic cases, and in only 4 (15 per cent) of the 27 non-toxic patients. When this tendency was first noted, it was believed to be due to increase in body weight following postoperative reduction of the basal metabolic rate. This explanation, however, is not supported

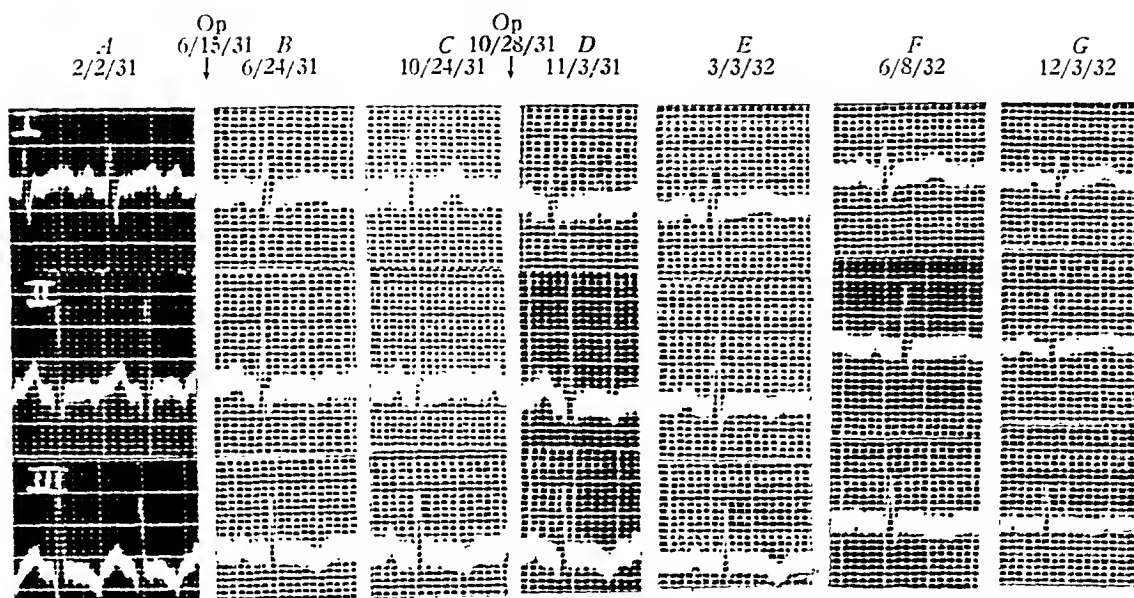


FIG. 1. ELECTROCARDIOGRAMS OF V.O., A WOMAN OF 23 YEARS, WHO HAD A TOXIC DIFFUSE GOITER, DURATION 6 WEEKS, BASAL METABOLISM BEFORE OPERATION (JUNE 14, 1931)  $+56$  PER CENT.

Right lobectomy June 15, 1931. Left lobectomy October 28, 1931. Last basal metabolism on August 10, 1932 was  $+18$  per cent.

This figure shows:

(1) Marked changes in T waves.  $T_2$  was inverted February 2, 1931, less inverted June 24, 1931, upright October 24, 1931, inverted once more November 3, 1931, less inverted March 3, 1932, upright June 8, 1932, upright December 3, 1932.

(2) Shift of the electrical axis to the left (patient gained 7 lbs.). Index on February 2, 1931 =  $-8$ . Index on December 3, 1932 =  $-3$ .

(3) P waves were large at first and subsequently reduced in size.

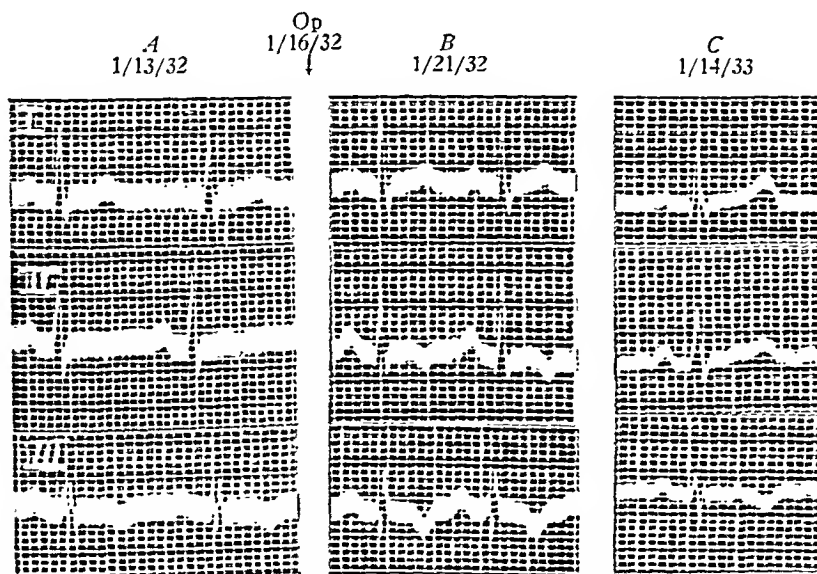


FIG. 2. ELECTROCARDIOGRAM OF B.S., A WOMAN OF 33 YEARS, WITH A NON-TOXIC NODULAR GOITER

Thyroidectomy was done on January 16, 1932.

This figure shows that marked changes may occur in the T waves of a non-toxic patient. On January 13, 1932,  $T_2$  was slightly inverted. On January 21, 1932, five days after operation,  $T_2$  was more deeply inverted. On January 14, 1933,  $T_2$  was normal. She had received no digitalis.

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Number of patients . . . . .	16	0	4	7	27	11	2	2	5	20	33	11	9	6	59	79
P wave 3 mm. or higher . . . . .				1	1	5				5	8	4	6	2	20	25
QRS complexes increased in size immediately after operation . . . . .					0					0	3	1			4	4
All QRS complexes smaller at last examination than at first . . . . .	1				1					0	6	3	1	1	11	11
Shift of electrical axis toward the left from first to last examination . . . . .	1		1	2	4	6	1	1	1	9	18	7	5	1	31	40
T waves 4 mm. or higher at first examination . . . . .	3			3	6	4	1		1	6	8	4		1	13	19
T waves increased in size immediately after operation . .			1		1			1	1	2	8	3	5	2	18	20
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|| Present at first examination in 4 cases.

¶ Present at first examination in 1 case.

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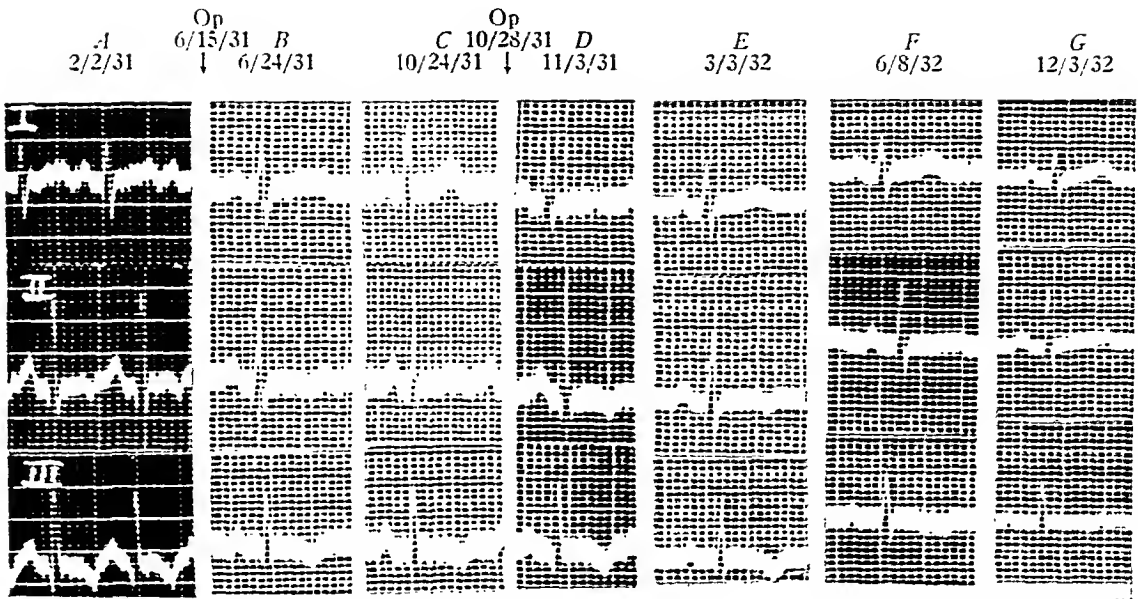


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Right lobectomy June 15, 1931. Left lobectomy October 28, 1931. Last basal metabolism on August 10, 1932 was  $+18$  per cent.

This figure shows:

(1) Marked changes in T waves.  $T_2$  was inverted February 2, 1931, less inverted June 24, 1931, upright October 24, 1931, inverted once more November 3, 1931, less inverted March 3, 1932, upright June 8, 1932, upright December 3, 1932.

(2) Shift of the electrical axis to the left (patient gained 7 lbs.). Index on February 2, 1931 =  $-8$ . Index on December 3, 1932 =  $-3$ .

(3) P waves were large at first and subsequently reduced in size.

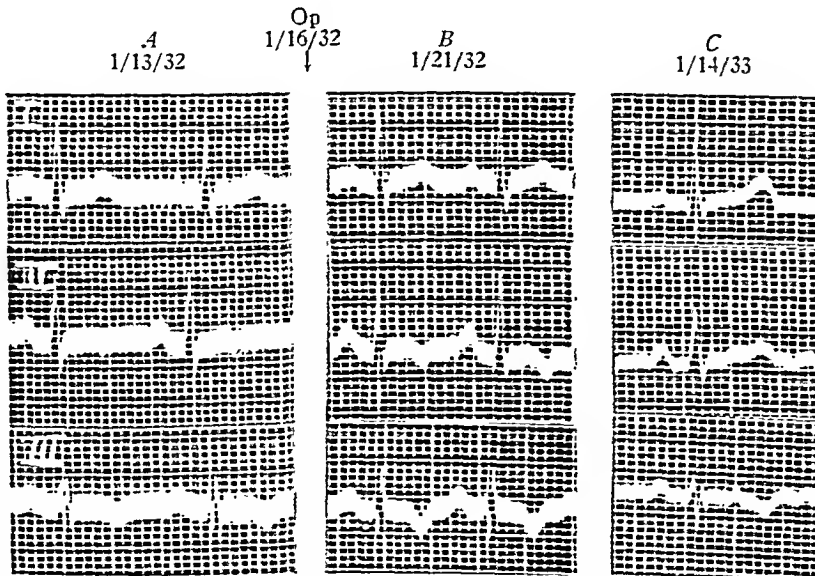


FIG. II. ELECTROCARDIOGRAM OF B.S., A WOMAN OF 33 YEARS, WITH A NON-TOXIC NODULAR GOITER

Thyroidectomy was done on January 16, 1932.

This figure shows that marked changes may occur in the T waves of a non-toxic patient. On January 13, 1932,  $T_2$  was slightly inverted. On January 21, 1932, five days after operation,  $T_2$  was more deeply inverted. On January 14, 1933,  $T_2$  was normal. She had received no digitalis.

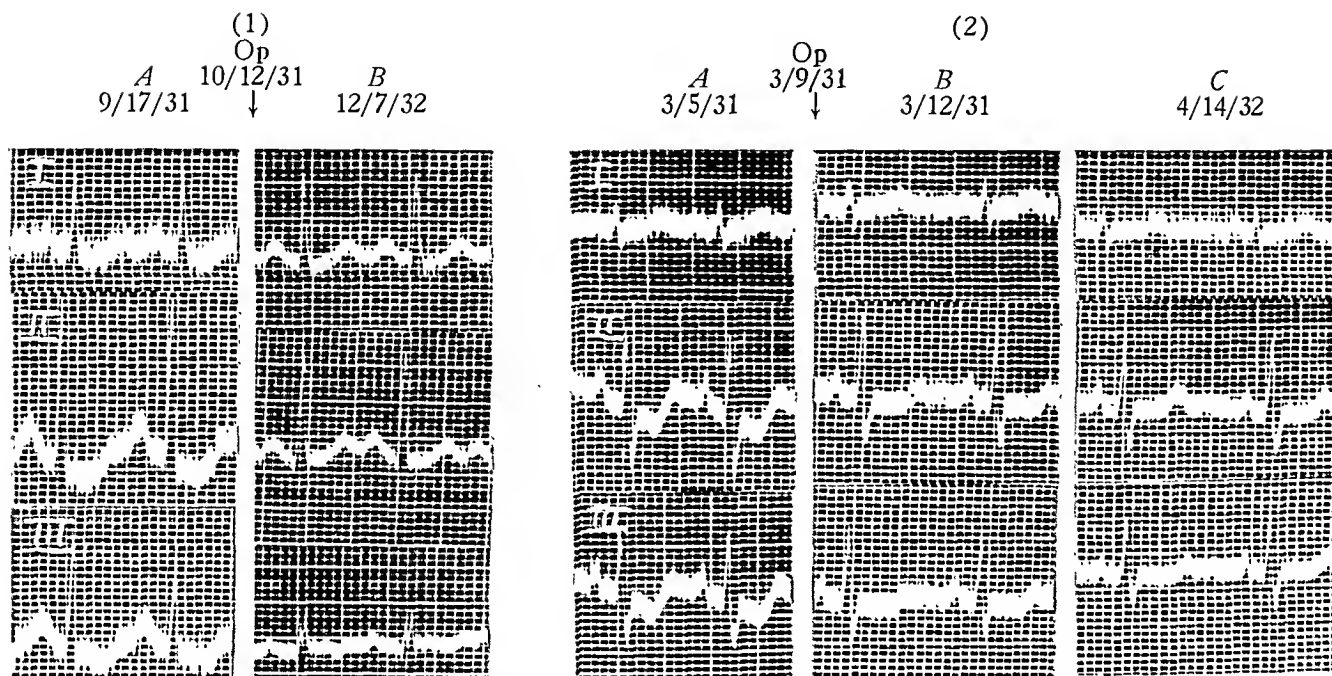


FIG. III. THIS FIGURE SHOWS R S-T INTERVAL DEVIATIONS IN (1) A TOXIC PATIENT AND IN (2) A PATIENT WITH A NON-TOXIC NODULAR GOITER.

(1) Electrocardiograms of J.R., a woman of 30 years, who had a toxic diffuse goiter, duration 4 years; basal metabolism before operation (September 22, 1931) + 71 per cent; basal metabolism 14½ months later (December 7, 1932), + 35 per cent.

*A.* First tracing September 17, 1931, heart rate 130; depression of the R S-T interval in all leads. The patient had received no digitalis. Immediately after operation (October 12, 1931) she was digitalized because of an attack of auricular fibrillation and developed deeply inverted T waves.

*B.* Last tracing December 7, 1932, heart rate 130. She had not received digitalis since shortly after her operation. The R S-T interval deviations had disappeared, despite the fact that the patient's thyrotoxicosis was only partially relieved by thyroidectomy.

In addition to the R S-T interval changes, this tracing shows (*a*) large P waves in the first tracing which became smaller in the last tracing and (*b*) a marked shift of the electrical axis toward the left in the last tracing, as compared with the first one, from -4 to +5. (The patient gained 30 lbs.).

(2) Electrocardiograms of M.McC., a woman of 37 years, who had a non-toxic nodular goiter, duration 12 years; basal metabolism before operation (March 4, 1931) + 14 per cent; one year later + 3 per cent.

The first tracing, *A*, taken on March 5, 1931 showed a depression of the R S-T interval. No digitalis had been administered. The change of R S-T interval had disappeared to some extent seven days later, *B*. It had vanished completely one year after operation, *C*. The patient gained 10 lbs. during the year. The electrical axis did not change.



by more careful analysis of the data. The cases were studied with reference to (a) the changes in axis, calculated on the basis of White's (15) index,<sup>2</sup> (b) the change in body weight, and (c) any cardiovascular or other conditions which might have influenced the electrical axis. Although the largest average gain of weight occurred in the group showing shift of the axis to the left, the change in the individual case was not necessarily proportional to change in weight. In the toxic group, for example, eight cases which showed gains of 10 lbs. or less showed a definite shift of electrical axis toward the left. On the other hand, six cases which gained from 30 to 50 lbs. showed either no change or a shift to the right. This change in axis was not accompanied by any characteristic changes in the T wave in Lead III. It cannot be correlated with changes in heart rate. Moreover the cases which failed to show this phenomenon were not necessarily those whose hyperthyroidism had not been relieved by operation. Many thyrotoxic patients show prominence and increased pulsation of the pulmonary artery, which tend to disappear when the thyrotoxicosis is relieved. This phenomenon, which suggests overaction of the right ventricle in thyrotoxicosis, may be related to the change of axis under consideration. However, a review of our cases, with this thought in mind, failed to elicit corroborative data. The presence and subsequent disappearance of prominence and increased pulsation of the pulmonary artery did not occur more frequently in those groups which showed a definite shift of electrical axis to the left. There may be some relationship between these two phenomena, but our methods failed to demonstrate it. We, therefore, feel that this shift in electrical axis, which so often accompanies surgical relief from hyperthyroidism, cannot be satisfactorily explained at present.

*S-T interval.* Four patients showed deviation of the S-T interval from the isoelectric line before operation—a depression in Leads II and III in each case. This abnormality disappeared in all instances before the first postoperative tracing was taken. There was no history of administra-

tion of digitalis prior to the first electrocardiogram in any of the 4 patients. The first patient had a non-toxic goiter; the second, a toxic nodular goiter; and the third, a toxic diffuse goiter; the final tracing was normal in each instance. The fourth patient had a toxic diffuse goiter; the final tracing showed isoelectric T waves in all leads. (The third patient was digitalized after operation and does not appear in the Table.)

*T waves.* (a) *Changes at the first and last examination.* Examination of Table I will show that our findings do not support the belief that the height of the T waves is directly proportional to the degree of thyrotoxicosis. Although 17 toxic patients showed smaller T waves at the last examination than at the first, 14 showed the reverse. Two patients with postoperative hypothyroidism developed small T waves, but similar changes occurred in 8 patients who did not show postoperative thyroid deficiency. Furthermore, in 10 patients who were incompletely relieved of their thyrotoxicosis, 3 had larger T waves at the last examination than at the first, 3 had smaller T waves, and 4 showed no change in size.

The incidence of T waves 4 mm. or more in height, in both the toxic and non-toxic groups, was about the same at the first and last examinations. T waves which had been normal before operation sometimes became abnormal at the last examination, and the reverse was also occasionally noted.

(b) *Immediate postoperative changes.* These likewise seemed to follow no definite rule. Seven non-toxic and 15 toxic patients showed slight reduction in the height of the T waves in the first postoperative tracing. Three non-toxic and 13 toxic patients showed a temporary reduction after operation, sufficient to be considered abnormal. However, one non-toxic and 20 toxic patients showed an increase in the amplitude of the T waves in the first postoperative tracing. This phenomenon was slightly more common in the group which had been toxic for 2 years or more (9 of 27 cases) than in the remainder of the toxic group (11 of 52 cases). Parade and Haas (16) report inversions of T waves immediately after operation in a series of cases of hyperthyroidism but stated that their patients received verodigen 0.5 gram and quinidin 0.2 gram three times daily from the fifth day before to the third day after

<sup>2</sup> The index of shift is the figure obtained by calculating the index according to White's formula for the first and last tracings of each individual patient and subtracting the former from the latter.



operation. Kämmerer and Obermaier (17) noted T wave negativity after thyroidectomy in 20 per cent of their (toxic?) cases. In order to evaluate this phenomenon properly it would be necessary to take daily tracings after operation, which we did not do. However, the time which elapsed between operation and the taking of the first postoperative tracing in our cases did not seem to be of importance in connection with the reduction of height of the T waves. We have not been able to find any satisfactory report of cases studied after other types of operation to serve as a control series. The fact that some patients with non-toxic goiter showed T wave changes similar to those seen in the toxic group casts some doubt upon the belief that the thyrotoxicosis itself is the determining factor.

The severity of the postoperative reaction, the type of anesthetic used, the presence of substernal extension of the thyroid, the occurrence of injuries to the recurrent laryngeal nerves, and the age and sex of the patients, all bore no apparent relation to the incidence of postoperative T wave inversion in our cases. Some patients showed such inversion after only one stage of a two-stage operative procedure. One individual showed an increase in size of T waves after her second operation but not after her first.

It thus appears that, while marked T wave changes may occur immediately after partial or subtotal thyroidectomy in hyperthyroidism, they are not all of the same type; their occurrence is at present unpredictable, and they follow no apparent pattern. Similar changes occur less frequently after the removal of non-toxic goiters.

(c) *Other changes.* A few patients showed T wave changes in addition to those noted above. Two toxic male patients, aged 27 and 32, developed inverted T waves in Lead I resembling "coronary" T waves, without any other evidence of coronary occlusion. In the first case this change appeared only in the last tracing, taken 18 months after operation. In the other, it appeared only in the tracing taken 4 months after operation and had disappeared 8 months later when the final examination was made.

Two female patients, aged 27 and 38, showed abnormal T waves throughout the period of observation with no other evidence of cardiovascular disease. The first had a toxic nodular goiter: T<sub>1</sub>

was isoelectric, T<sub>2</sub> and T<sub>3</sub> inverted throughout. (This patient was followed for only seven months after operation and does not appear in the Table.) The second patient had a toxic diffuse goiter: the preoperative tracing showed T<sub>1</sub> isoelectric, T<sub>2</sub> and T<sub>3</sub> inverted. The last tracing, taken 2 years after operation, showed T<sub>1</sub> upright, T<sub>2</sub> diphasic, and T<sub>3</sub> inverted.

Two female patients, thyrotoxic and desperately ill on admission, with congestive heart failure and auricular fibrillation, showed normal electrocardiograms one year after operation. All evidence of cardiovascular disease had disappeared. The first and second tracings may have been influenced by digitalis administration during the preoperative period and cannot be accurately interpreted.

Only 3 patients in the toxic group showed no electrocardiographic changes whatever throughout the entire period of observation. One of these patients had an enlarged heart and showed auricular fibrillation throughout. Despite digitalization the T waves remained upright and unchanged in size or appearance.

It is possible that the T wave in thyrotoxic persons is subject to more frequent changes in size and shape and is more labile than in other individuals. Marked changes may appear in normal persons as a result of exercise. Thus an upright T wave may become higher immediately after exercise and be inverted ten minutes later (18). It is possible that in thyrotoxicosis with instability of the vegetative nervous mechanism the T wave may be more readily altered by psychic and physical disturbances. Further investigation is necessary to support this suggestion. Whether or not this explanation is valid, the fact remains that T wave inversion appears quite frequently as a transient phenomenon in patients with thyrotoxicosis. Consequently in this disease caution is necessary in interpreting the significance of inverted T waves. T wave abnormalities in patients with thyrotoxicosis do not necessarily indicate the presence of chronic myocardial disease.

We have been unable to find any significant relationship between electrocardiographic changes and variation in heart size in our patients. In the toxic group the smaller variations in heart size occurred without any characteristic electrocardiographic accompaniment. Those patients who showed more marked variations in size had

usually been in congestive failure and hence had received digitalis at some time during the period of observation; in them interpretation of the electrocardiograms was not possible. Most of these patients are not included in the analysis of the orthodiagraphic data. In the non-toxic group enlarged hearts were usually associated with other cardiovascular abnormalities, thus making interpretation of the tracings difficult.

Likewise no relation could be found between electrocardiographic changes and (a) variation in heart rate, (b) the degree of relief from thyrotoxicosis obtained by operation, (c) the duration or severity of the thyrotoxicosis, or (d) the age of the patients.

An attempt was made to determine the prognostic value of the electrocardiogram in patients who were to be subjected to thyroidectomy. The records of all patients who died in the hospital on all surgical services after thyroidectomy, from January, 1931, to October, 1934, were studied. There were ten such patients. Seven of these had electrocardiograms taken during the month preceding operation; all were thyrotoxic. Of these 7 cases, 5 had tracings which were within normal limits except for simple tachycardia. The sixth patient showed auricular fibrillation, the QRS and T waves being normal. The seventh showed diphasic T waves in Leads I and II.

Three of the 137 patients in our series died during the period of observation, after leaving the hospital, none as a direct result of operation. One died of "edema of the larynx" three months after operation; her electrocardiogram was normal except for transient postoperative auricular fibrillation. One died two months after operation of unknown cause; he had had auricular fibrillation throughout with flat T waves and numerous ventricular extrasystoles. The third patient (who had been in auricular flutter on admission) died one year after operation of carcinoma of the liver.

Although these facts are rather meager, they do not suggest that routine electrocardiograms *per se* have any value in determining whether thyrotoxic patients will live through (a) the immediate postoperative period or (b) the year following thyroidectomy.

Finally, we were unable to discover any tendency of the various changes in P waves, QRS

complexes or T waves, listed in Table I, to group themselves in any characteristic fashion in individual patients.

#### SUMMARY

Electrocardiographic studies have been made in 102 thyrotoxic patients and 35 patients with non-toxic goiter. These studies were made before partial or subtotal thyroidectomy and at successive intervals thereafter, up to one year in most instances. The records of 8 patients with non-toxic goiter and 27 with hyperthyroidism have been excluded from our analysis. Our findings have been analyzed with respect to the following: (1) the frequency of abnormal electrocardiograms before operation and the effect of operation upon them; (2) the occurrence of changes in the P waves, P-R interval, QRS complexes, electrical axis, S-T interval and T waves; the nature of these changes; and the effect of operation upon them; (3) the incidence of abnormalities of cardiac rhythm and the effect of operation upon them; (4) the relation between electrocardiographic abnormalities and (a) variations in size of the heart, (b) the presence of substernal goiter, (c) variation in heart rate, (d) duration and severity of thyrotoxicosis, (e) severity of postoperative reaction, (f) type of anesthesia employed, (g) injury to the recurrent laryngeal nerve, (h) degree of relief of thyrotoxicosis obtained by operation, (i) age, (j) sex and (k) the state of cardiac compensation.

#### CONCLUSIONS

1. Abnormal electrocardiograms occur with about the same frequency in patients with toxic and non-toxic goiter (45 and 41 per cent respectively in our series).

2. Changes in normal electrocardiograms after partial or subtotal thyroidectomy are more common in patients with hyperthyroidism than in those with non-toxic goiter (97 per cent as compared with 56 per cent in our series).

3. Large P waves (3 mm. or more in height) are common in hyperthyroidism. They are usually reduced to less than 3 mm. after successful thyroidectomy.

4. Following partial or subtotal thyroidectomy in hyperthyroidism, the electrical axis of the heart shifts to the left in about half the cases.

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3. Large P waves (3 mm. or more in height) are common in hyperthyroidism. They are usually reduced to less than 3 mm. after successful thyroidectomy.

4. Following partial or subtotal thyroidectomy in hyperthyroidism, the electrical axis of the heart shifts to the left in about half the cases.

The cause of this shift is unknown; it is apparently not due to changes in body weight alone.

5. Marked T wave changes occur before and after thyroidectomy in hyperthyroidism, but they follow no apparent pattern, and their occurrence is unpredictable. The T waves in hyperthyroidism are not characteristically larger than normal, nor do they necessarily become reduced in size when the hyperthyroidism is relieved.

6. T wave inversion in thyrotoxicosis does not necessarily indicate the presence of chronic myocardial disease. It occurs quite frequently as a transient phenomenon.

7. The electrocardiographic changes seen in patients with toxic and non-toxic goiter do not seem to be related to (a) changes in heart size, (b) heart rate, (c) postoperative improvement in thyrotoxicosis, (d) duration or severity of thyrotoxicosis, (e) age, (f) sex, (g) the state of cardiac compensation, (h) the presence of substernal goiter, (i) operative injuries to the recurrent laryngeal nerve, (j) the type of anesthesia employed or (k) the severity of the postoperative reaction.

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# STREPTOCOCCAL AGGLUTININS AND ANTISTREPTOLYSINS IN RHEUMATOID (ATROPHIC) ARTHRITIS

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Numerous attempts during the past several years to isolate bacteria of possible etiologic significance from the blood, synovial fluid, or tissues of patients with rheumatoid (atrophic) arthritis have yielded inconsistent results (1). In general, the lack of uniformity in the results of bacteriologic studies in this disease is striking.

Nevertheless, a suggestion of a possible relationship of hemolytic streptococci to rheumatoid arthritis was offered by the demonstration by Nicholls and Stainsby (2) of agglutinins for hemolytic streptococci in high titer in the blood serum of a majority of patients with this disease. Several subsequent reports (3-8) confirm in general the results of Nicholls and Stainsby, and show that the tendency of hemolytic streptococci to be agglutinated by sera from cases of rheumatoid arthritis is not restricted alone to the "typical strain" of Cecil, Nicholls, and Stainsby (9), but that the reaction is characteristic of *beta* (hemolytic) streptococci as a group.

Following infection by hemolytic streptococci, the blood serum contains streptococcal antihemolysin (antistreptolysin) in high titer. This was demonstrated by Todd (10) and later confirmed by Coburn and Pauli (11), working in conjunction with Todd, and by Myers and Keefer (12), in such infections as acute follicular tonsillitis, scarlet fever, erysipelas, and acute rheumatic fever. Wilson, Wheeler, and Leask (13) reported that following streptococcal infections the average antistreptolysin titer was definitely higher than in normal subjects. Seegal and Lyttle (14) found high antistreptolysin titers in a large percentage of a series of cases of acute glomerular nephritis.

Inasmuch as the demonstration of agglutinins for hemolytic streptococci in the serum of patients with rheumatoid arthritis is at least suggestive of some relation of these organisms to this disease, it appeared that an investigation of the antistreptolysin content of sera from patients with

rheumatoid arthritis might be of interest. Myers and Keefer (12), whose paper appeared soon after the inception of this work, claimed that "the sera of patients with rheumatoid, as well as other forms of arthritis, fell into a group which resembled that of normal individuals or patients with non-streptococcal infections."

It is the purpose of this communication, first, to add confirmation of the presence of agglutinins for hemolytic streptococci in high titer in the sera of patients with rheumatoid arthritis, and second, to demonstrate that a certain percentage of these sera contain antistreptolysin in titers above the normal range.

## AGGLUTINATION TESTS

*Cases studied.* Agglutination tests were performed on 69 sera from 62 cases of typical rheumatoid arthritis. The patients in this group presented the typical syndrome of a chronic polyarthritis which tended to progress to ankylosis and deformity. All degrees of severity of the disease were included in the series. The duration of the disease ranged from 3 months to 36 years; the great majority of the cases were of one year's duration or over. The age of the patients, including two children with Still's disease, ranged from 6 to 68 years; three-quarters of the group were under 50 years of age, and about half of the group were in the fourth and fifth decades.

As controls, 129 sera from 125 persons presenting a variety of infectious and non-infectious, orthopedic, medical, and surgical conditions were employed. These included hypertrophic arthritis, spondylitis ankylopoietica (of the Marie-Struempell or Bechterew type), gonorrheal arthritis, osteomyelitis, rheumatic fever, proven infections by hemolytic streptococci, and miscellaneous other conditions. The age distribution of the control series was similar to that of the patients with rheumatoid arthritis.

TABLE I  
*Agglutination by sera from cases of rheumatoid arthritis*

Case number	Sex and age	Hemolytic streptococci					<i>Streptococcus viridans</i> 208	Staphylococci		Anti-streptolysin titer units per cc.	Sedimentation rate mm. in 45 minutes	Duration of arthritis
		"Typical strain"	Rheumatic fever	Erysipelas	Erysipelas	Scarlet fever		<i>Micrococcus deformans</i>	<i>Staphylococcus aureus</i>			
		AB66	Q33	21	B3	C203						
1	F38	2560	320		320	640	—			190	81	3 months
		2560	320		640	640	—			190		
2	M26	1280	1280		—	40	—			571	43	6 months
		1280	1280		—	40	10			380		
3	F40	1280	1280		80	640	—			190		3 years
4	F48	1280	1280		80	40	—			190		8 years
5	F50	1280	640	160			—	320	640		90	5 years
6	F27	1280	640	80			20	160	—			3 years
7	F55	1280	640	40			—	80	160			8 years
8	F25	1280	640		160		—			95	33	5 years
9	M25	1280	640		10	160	—			95	50	2 years
		320	640		—	—	—			47	45	
10	F57	1280	640		320	1280	—			95	93	3 years
11	F38	1280	320		160	160	—			380		5 years
12	F36	1280	320		80	80	—			95		22 years
13	M42	1280	320		320	320	—			47	40	3 years
14	M34	1280	160		20	—	—			95	42	18 months
15	F40	1280	40		—	320	—			190	11	1 year
16	M46	1280	320		320	—	—			95	79	6 years
17	F 7	1280	—		—	—	—			95		4 years
18	F26	1280	160		—	—	—			<47		7 months
19	M51	640	640	—			—	—	—		33	4 years
20	F 6	640	320		40	20	—			95	20	3 years
21	M54	640	160		640		—			47	54	4 years
22	F27	640	320	40			—	10	40		40	6 years
23	M39	640	320		320		—			47	72	1 year
24	F30	640	320	—			10	—	—			5 years
		320	320		320	—	—			95		
25	M37	640	320		—	40	—			190	50	1 year
26	F50	640	320	20			—	40	40		56	2 years
27	M21	640	320		160	320	—			380	10	2 years
		640	640		320	320	—			285	13	
		640	320		40	160	—			95	15	
		1280	640		160		—			95		
28	M54	640	320	—			—	—	40			5 months
29	F42	640	320		—	80	—			95	17	2 years
30	F45	640	320		40	640	—			47		1 year
31	M24	640	80		—	—	—			380	75	3 years
32	M51	640	80	—			20	160	—			1 year
33	M44	640	—		40	—	—			47	65	3 months
34	F28	320	640		80	80	—			47	10	3 months
35	F56	320	640	—			—	—	640			4 years
36	M35	320	320		80		—			95	18	12 years
37	F60	320	320	80			—					36 years
38	F52	320	160		160		—			47	35	19 years
39	M41	320	320		40	10	—			47	45	4 years
40	F44	320	160		160		—			190	70	3 years
41	F49	320	320	80			—	—	—			12 years
42	F20	160	640		—	10	20			210	47	6 months
43	F40	160	640		20	1280	—			190	19	3 months
44	M49	160	320	—			—	—	—		24	5 years
45	F62	160	320		—	—	—			47		20 years
46	F38	160	320		20	—	80			952	20	2 years
47	F68	160	160	40			—	—	—			10 years
48	F39	160	80		40	40	—			<47	90	5 months
49	F19	160	80		—	—	—			47	25	4 years
50	F49	160	80		20	—	—			95	33	1 year
51	M39	160	80		40	40	—			47		6 months
52	F42	80	—	—			—	—	—			4 years
53	F61	40	640		40	320	—			28	82	2 years



TABLE I (Continued)

Case number	Sex and age	Hemolytic streptococci					<i>Strepto- coccus viridans</i>	Staphylococci		Anti- strepto- lysin titer	Sedimen- tation rate	Duration of arthritis
		"Typical strain"	Rheu- matic fever	Erysi- pe- las	Erysi- pe- las	Scarlet fever		<i>Micro- coccus de- formans</i>	<i>Staphylo- coccus aureus</i>			
		AB66	Q33	21	B3	C203						
54	F57	40	80		20	20	—			47		7 years
55	F54	40	80		160	40	—			0		3 years
56	M47	—	160		—	—	—			133	65	10 years
57	F67	—	—		—	—	—			190		9 months
58	M50	—	—	—			—				20	3 months
59	M18	—	—	—			—	—	1280			3 months
60	F47	—	—		—	—	—			<47	8	7 years
61	F34	—	—		—	640	—			95		4 years
62	F22	—	—	—			—	80	320		38	5 years

The clinical diagnosis of Cases Number 17 and 20 is Still's disease. In the instances where more than one sample of serum was obtained, the time intervals between taking of the samples was as follows: Number 1—20 days; Number 2—2½ months; Number 9—4½ months; Number 24—12 months; Number 27—3 months, 5½ months, and 4½ months.

### Technic of agglutination tests

The organisms employed in the agglutination tests were:

#### Hemolytic streptococci:

"AB66"—Cecil's "typical strain," isolated from the blood of a patient with rheumatoid arthritis.

"Q33"—a rheumatic fever strain, "from the tonsillar exudate of a patient who had suffered for months from severe polyarthritis and carditis with congestive failure" (15).

"21"—from a case of erysipelas.

"B3"—from a case of erysipelas.

"C203"—from a case of scarlet fever.

#### *Streptococcus viridans*:

"208"—isolated in this laboratory from the blood in a typical case of subacute bacterial endocarditis.

#### Staphylococci:

"*Micrococcus deformans*"—isolated by Crowe from the urine of a patient with rheumatoid arthritis.

*Staphylococcus aureus* "182"—isolated in this laboratory from the blood stream in a fatal case of infected sinus thrombosis.

For their courtesy in supplying certain of these strains, our thanks are due to Dr. W. J. Stainsby, for "AB66"; to Dr. H. F. Swift, for "Q33"; to Dr. K. E. Birkhaug, for "21"; to Dr. M. H. Dawson, for "B3" and "C203"; and to Dr. H. W. Crowe, for *Micrococcus deformans*.

The sera used for the tests were obtained under aseptic precautions and kept in sterile tubes at 4° C. All of the sera were tested with Cecil's "typical strain," Q33, one of the erysipelas strains, and *Streptococcus viridans*. The scarlet fever strain, C203, was used in agglutination tests with

114 sera, and the two staphylococcus strains with 80 sera.

In about half of the agglutination tests, living 24-hour cultures in bacto-heart-infusion broth were employed. Subsequently, younger cultures (16 to 20 hours old) in "streptolysin broth" were used. This medium was made according to the formula described by Swift and Hodge (16) and was found to give excellent diffuse growth. A small series of tests was also made with heat-killed suspensions in "streptolysin broth." Equally good results were obtained with antigens prepared by all three methods. The suspensions were diluted with physiological salt solution to the desired turbidity.

Serial dilutions of the sera were made in serological tubes with physiological salt solution, and an equal volume of bacterial suspension was added to each tube, to give final dilutions ranging from 1:10 to 1:1280. The tubes were incubated in a water bath at 56° C. for two hours, then transferred to the refrigerator, at 4° C. The titer was read after holding the tubes in the refrigerator overnight. The last dilution showing definite clumping of the organisms was taken as representing the agglutination titer.

### Results of agglutination tests

*Rheumatoid arthritis.* The results of agglutination tests with sera from cases of rheumatoid arthritis are shown in Table I. Included in the table, for comparison, are the antistreptolysin



titers and sedimentation rates.<sup>1</sup> Sixty-nine sera from 62 patients were employed in the tests. Of these sera, 59 or 84 per cent (representing 82 per cent of the patients) caused agglutination of Cecil's "typical strain" streptococcus (AB66) in a titer of 1:160 or higher. With few exceptions, agglutination of AB66 was accompanied by agglutination of the streptococcus from rheumatic fever (Q33), and nearly one-fifth of the sera gave a higher titer with Q33 than with AB66. A total of 60 (87 per cent) sera (representing 85 per cent of the patients) caused agglutination of AB66 or Q33, or both, in a titer of 1:160 or higher. The trend of the agglutination titers obtained with the erysipelas and scarlet fever strains was generally appreciably lower than the titers obtained with the "typical strain" or with Q33.

Six of the sera caused agglutination of the strain of *Streptococcus viridans* used, the highest titer obtained being 1:80.

In contrast to the large number of high agglutination titers obtained in the group of rheumatoid arthritis, are the many negative tests obtained with the sera of these "control" groups. When agglutination occurred, the titers obtained with Q33 usually ran parallel to the titers obtained with AB66, and the scattered agglutination titers obtained with the other hemolytic streptococci were generally appreciably lower.

It may be noted that included among the controls are sera from six cases of spondylitis ankylopoietica (of the Marie-Struempell or Bechterew type) and from sixteen cases of hypertrophic arthritis or arthritis of the sacro-iliac articulations. Sera from three cases of spondylitis ankylopoietica and from three cases of hypertrophic arthritis agglutinated AB66 or Q33, or both, in dilutions of from 1:160 to 1:1280. It is recognized that while the joints of the spinal column are sometimes affected in rheumatoid arthritis, all cases

TABLE II

Summary of agglutination of Cecil's "Typical strain" streptococcus (AB66) and of Q33

	Number of individuals	Total number of sera	Number of sera causing agglutination in various dilutions								
			Negative	1:10	1:20	1:40	1:80	1:160	1:320	1:640	1:1280 and over
Rheumatoid arthritis.....	62	69	6	0	0	0	3	6	10	23	21
Chronic arthritides other than rheumatoid arthritis.....	31	32	15	1	0	0	2	1	6	4	3
Miscellaneous infectious and non-infectious orthopedic conditions.....	35	35	29	0	0	0	1	0	3	1	1
Osteomyelitis.....	14	14	8	1	0	1	0	0	1	0	3
Miscellaneous medical and surgical conditions....	11	11	9	0	1	0	1	0	0	0	0
Rheumatic fever.....	24	25	8	0	0	2	1	2	4	5	3
Infections by hemolytic streptococci.....	5	7	1	0	0	0	0	0	2	4	0
Normal persons.....	5	5	2	0	0	0	1	2	0	0	0

Seventeen of the sera were employed in agglutination tests with the two strains of staphylococci. Seven contained no agglutinins for these organisms, and ten caused agglutination of one or both strains, the titers for *Micrococcus deformans* ranging from 1:10 to 1:320, and for *Staphylococcus aureus* from 1:40 to 1:640.

"Control" groups. A summary of the results of agglutination tests with sera from the various disease groups other than rheumatoid arthritis, and from normal individuals, appears in Table II.

of spondylitis ankylopoietica need not have a rheumatoid basis. Furthermore, while cases of the hypertrophic type are generally of a non-infectious origin (idiopathic hypertrophic arthritis), it is known that damage to the tissues of a joint by a previous infection may occasionally be the basis for the development of hypertrophic joint changes.

We obtained a somewhat larger percentage of high agglutination titers in our series with sera from cases of rheumatic fever than has been reported by others. High titers were obtained with the various hemolytic streptococci and sera from cases of proven infection by hemolytic streptococci.

<sup>1</sup> The sedimentation rate was determined according to the technic described by Weiss (17).

Of the 129 sera from cases other than rheumatoid arthritis, only 7 caused agglutination of *Streptococcus viridans*. These were: 1 bronchial asthma (1:10); 1 gout (1:80); 1 chronic osteomyelitis (1:20); 1 rheumatic fever (1:40); 1 chorea (1:40); 2 proven infections by hemolytic streptococci (1:10 and 1:20).

About half of all the control sera which were tested against the two strains of staphylococci caused agglutination of one or both of the strains. The majority of the titers ranged from 1:10 to 1:80, although occasionally titers ranging up to 1:1280 were obtained.

#### *Agglutination by synovial fluid*

Nicholls and Stainsby (2) stated that they performed agglutination tests with "typical strain" streptococci and synovial fluid from three cases of chronic infectious arthritis. The agglutination titers obtained were 1:640, 1:2560, and 1:5120, respectively.

In our series, forty-four specimens of synovial fluid, from a variety of infectious and non-infectious arthropathies, were used in agglutination tests. The technic was the same as that for the sera, except that the final dilutions ranged from 1:5 to 1:160. In the event that agglutination occurred throughout the series, a second test was run, using dilutions up to 1:5120.

Twenty of the synovial fluids were from patients who had also furnished blood serum for agglutination tests. A comparison of the agglutination titers of the fluids and their corresponding sera is found in Table III. The source of the fluids is as follows: rheumatoid arthritis—5; gonorrheal arthritis—3; hypertrophic arthritis—3; chronic synovitis—3; tuberculous synovitis—2; luetic synovitis—1; miscellaneous conditions of joints—3.

The titers of three of the five fluids from rheumatoid arthritis were 1:160, 1:640, and 1:5120, respectively. The homologous sera in all three

TABLE III  
*Agglutination by serum and synovial fluid from same patient*

Serial number	Hemolytic streptococci						<i>Streptococcus viridans</i>		Staphylococci				Clinical diagnosis
	AB66		Q33		21		208		<i>Micrococcus deformans</i>		<i>Staphylococcus aureus</i>		
	Serum	Synovial fluid	Serum	Synovial fluid	Serum	Synovial fluid	Serum	Synovial fluid	Serum	Synovial fluid	Serum	Synovial fluid	
15	1280	640	160	80	40	20	—	—	—	—	—	—	Rheumatoid arthritis
29	640	5120	320	640	—	40	—	—	—	—	40	40	
45	160	160	320	80	—	5	—	—	—	80	—	320	
60	—	—	—	—	—	—	20	40	10	—	1280	80	
63	—	—	—	—	—	—	—	—	80	—	320	80	
67	640	—	640	160	—	—	—	—	20	—	160	160	Chronic arthritides other than rheumatoid arthritis
68	10	—	10	—	—	—	—	—	40	—	—	—	
69	—	—	—	—	320	—	—	—	10	—	—	—	
70	640	160	640	160	40	—	—	—	20	—	1280	160	
71	320	40	320	10	—	—	—	—	10	—	320	80	
72	—	—	—	—	—	—	—	—	—	—	320	80	
73	—	—	—	—	—	—	—	—	—	—	—	—	Chronic synovitis
74	—	—	—	—	—	—	—	—	—	—	—	—	
75	—	—	—	—	—	—	—	—	80	—	—	—	
76	—	—	—	—	—	—	—	—	40	—	—	—	Luetic synovitis
77	—	—	—	—	—	—	—	—	80	—	—	—	Tuberculous synovitis
78	—	—	—	—	40	160	—	—	40	—	—	160	
79	—	—	—	—	—	—	—	—	—	—	—	—	Internal derangement semi-lunar cartilage
80	—	—	—	—	—	—	—	—	40	—	—	—	Prepatella bursa
81	—	—	—	—	—	—	—	—	80	—	160	160	Lipoma of knee

cases contained agglutinins in significant titer. Two synovial fluids and their corresponding sera contained no agglutinins for hemolytic streptococci.

A total of five of the 44 fluids caused agglutination of Cecil's "typical strain" streptococcus. Three of these were the fluids from rheumatoid arthritis described above; the other two were from cases of hypertrophic arthritis. Scattered agglutinations of Q33 and of an erysipelas strain occurred, usually in low titer, although three titers of 1:160, and one of 1:640 were obtained. These titers occurred with fluids from cases of gonorrheal arthritis and tuberculous synovitis (1:160) and from one case of hypertrophic arthritis (1:640). *Streptococcus viridans* was agglutinated only once—by a dilution of 1:40 of a synovial fluid from a case of rheumatoid arthritis; this fluid caused agglutination of no other streptococcus, but agglutinated *Staphylococcus aureus* in a dilution of 1:80.

It is interesting to note that 22 of the 44 fluids caused agglutination of *Staphylococcus aureus*. The titers of seven fluids were 1:40 or 1:80; the titers of fourteen fluids were 1:160, and one had a titer of 1:320. When both fluid and serum from the same patient were tested, agglutination occurred with both, with few exceptions, and usually the titer of the serum was higher than that of the fluid. Agglutination of *Staphylococcus aureus* occurred with some synovial fluids from all of the disease groups represented.

#### *Correlation between agglutination titer and certain phases of rheumatoid arthritis*

Several attempts have been made to correlate the agglutination titer with various features of the disease (2, 3, 4, 6), such as its duration, number of joints involved, age of the patient, etc. We were unable to establish a correlation of the agglutination titer with any clinical aspects of the disease. We also found no correlation of the agglutination titer with the sedimentation rate, in confirmation of the reports of Dawson, Olmstead, and Boots (4) and of Keefer, Myers, and Oppel (5).

#### *Comment*

It appears to be reasonable to assume that an agglutination titer of 1:160 or higher is "signifi-

cant," or indicative of infection by streptococci. This titer was considered to be of significance in rheumatoid arthritis by Gray and Gowen (3) and by Dawson (4), and their associates, while a significant titer of 1:320 was adopted by Nicholls and Stainsby (2), and by Cox and Hill (6). A titer of 1:160 is much higher than the titers which are commonly accepted as being diagnostically significant in such infections as typhoid fever, Brucellosis, and typhus fever.

The fact is well established that sera from the majority of patients with rheumatoid arthritis contain agglutinins in high titer for hemolytic streptococci, a finding which can hardly be explained as entirely fortuitous. Furthermore, sera from arthritic conditions other than rheumatoid arthritis do not give such consistently high titers.

Nicholls and Stainsby consider that the agglutination in high titer of their "typical strain" streptococcus by sera from cases of rheumatoid arthritis "appears to be a true immunological response," and that the results they have reported "lend strong support to the theory that the 'typical strain' streptococcus is an important etiologic factor" in this disease. Dawson and his associates concluded that, while many of the features of the reaction are indicative of a true immunological response, the results obtained could be considered only as suggestive of an association of hemolytic streptococci with rheumatoid arthritis. Further suggestive evidence has been adduced, they believe, by their comparative study of agglutination and precipitation reactions, in which "a close approximation, but not an absolute agreement" is obtained in the results of the two tests.

It appears to be satisfactorily demonstrated that *beta* type streptococci, particularly such organisms as Cecil's "typical strain," NY5, or Q33, are characteristically agglutinated by sera from patients with rheumatoid arthritis. Cox and Hill (6) found that no other organism which they used had as great a serologic specificity for sera from cases of atrophic arthritis as did Cecil's "typical strain." However, they employed only one other strain of hemolytic streptococcus (isolated from the stool of a patient with rheumatoid arthritis), and they discarded another strain (NY5) after a few tests, since it always gave parallel agglutinations with Cecil's streptococcus, but in a lesser degree. They felt that definite deductions as to the etiologic

rôle of Cecil's "typical strain" would be premature. Wainwright (7), who used Cecil's "typical strain" and NY5, concludes that a positive agglutination "does not indicate of necessity a causal relationship between hemolytic streptococci and rheumatoid arthritis but it does suggest that streptococci play a rôle in this disease . . . the frequency with which it [agglutination] occurs in rheumatoid arthritis is the most incriminating evidence thus far produced against the streptococcus in this disease and merits some consideration."

The assumption that the reaction is a true immunological response suggests that it can be used for diagnostic purposes. Gray and Gowen (3) believe that it "is undoubtedly of considerable value in differential diagnosis, particularly in osteoarthritis." Cox and Hill (6), on the other hand, point out that "ordinarily a laboratory procedure is unnecessary in establishing a diagnosis of arthritis," and feel, from the results of their series of tests, that while the test may be used occasionally in doubtful cases and under carefully controlled conditions, it is of doubtful value in prognosis or as an isolated laboratory procedure.

The agglutination reaction with hemolytic streptococci apparently can serve to confirm a clinical diagnosis of rheumatoid arthritis, but its use hardly seems necessary in the majority of cases, where the clinical signs and symptoms render the diagnosis quite apparent. However, in borderline cases which are difficult of clinical diagnosis, the frequency of high titers in rheumatoid arthritis, and the comparative infrequency of similar titers in other types of arthritis, make it possible to indicate a *probable diagnosis*, based upon the results of agglutination tests with hemolytic streptococci, such as Cecil's "typical strain" or a strain giving closely parallel results.

#### ANTISTREPTOLYSIN TESTS

In testing sera for their antistreptolysin content, Todd (10) titrated against a unit expressed in terms of the minimal hemolytic dose of streptolysin. Inasmuch as streptolysin produced by hemolytic streptococci of human origin is subject to reversible oxidation and reduction, and the filtrate is hemolytic only when in the reduced state, the hemolytic power of a given filtrate may not remain constant.

However, it has been demonstrated by Hodge and Swift (18) that under certain conditions the power of streptolysin to combine with antisera remains constant over a considerable period. Their method of titrating antisera in terms of the "constant combining power" of streptolysin thus assures a reasonable degree of accuracy and obviates the necessity of having to resort to repeated determinations of the hemolytic titer.

#### *Technic of antistreptolysin tests*

The streptolysin used throughout the tests was prepared from cultures of the scarlet fever strain of hemolytic streptococcus, C203, which was used in the agglutination tests described above.

The technic used was that described by Swift and Hodge for preparing streptolysin (16), and by Hodge and Swift for titrating the minimal hemolytic dose and combining power of streptolysin, and for determining the antistreptolysin content of serum in terms of the constant combining power of streptolysin (18).

Without going into the details of the technic, a few pertinent facts may be recorded. Streptolysin was reduced *in vacuo* for 2 to 3 hours with 0.1 per cent of freshly ground sodium hydrosulfite, immediately after the 16 to 17 hour "streptolysin broth" culture had been filtered through a Seitz filter. The reduced streptolysin was immediately dispensed in sterile tubes or flasks, covered with a thick layer of sterile vaseline, and stored in the refrigerator.

Rabbit erythrocytes were used in all of these tests, as the red blood cells of other animal species have been found to give somewhat inconsistent results.

Streptolysin is "standardized" in terms of its *constant combining power* by determining the amount of streptolysin which is just inhibited by one antistreptolysin unit of a previously titrated serum of known antistreptolysin content.

Serum is titrated for its antistreptolysin content by determining that serum dilution which just inhibits hemolysis of 0.5 cc. of a 5 per cent suspension of twice washed rabbit erythrocytes by one combining unit of standardized streptolysin after incubation at 37° C. for one hour. The antistreptolysin titer of a serum is recorded as

the reciprocal of that dilution which just prevents hemolysis, as described above.

### *Standards*

In tests of this type it is obvious that standards should be established for the titration of streptolysin and antistreptolysin, so that the results obtained in various laboratories may all be recorded in similar terms. Only by this means may adequate comparisons be made of published reports from different laboratories. Recognizing this fact, Todd (19) has set aside a quantity of serum of known antistreptolysin potency, to be used as an arbitrary standard in antistreptolysin determinations.

The streptolysin of Hodge and Swift was standardized "with a number of sera furnished by Dr. Coburn . . . duplicates of these sera had been tested by Todd" (18). Through the courtesy of Dr. Hodge, samples of serum and of standardized streptolysin were obtained, and against these our own streptolysin and a few sera were standardized in a preliminary series of titrations. Recently, Dr. Todd kindly sent us some of his standard antistreptolysin serum which was used in a series of comparative tests with sera whose original titrations refer back to the standards supplied by Dr. Hodge. Thus checks on our streptolysin were obtained directly against Todd's standard serum, and indirectly against his standard, by means of serum supplied by Dr. Hodge.

*Antistreptolysin titer of normal serum.* In order to evaluate the results of antistreptolysin determinations, it is essential to know to what extent antistreptolysin is present in the serum of normal individuals, and to establish a normal value.

Todd (10), and Coburn and Pauli (11) have demonstrated that the maximum antistreptolysin titer of the serum of normal individuals is about 100 units per cubic centimeter. Parallel tests with Todd's serum and with sera which were titrated by the method of Hodge and Swift have shown that the value which we obtained for normal individuals is essentially equivalent to the value obtained by Todd. It would appear that, for the sake of exact comparison with other published results, the titers of antisera should be expressed in terms of some arbitrary standard, such as is represented by the serum which has been made

available by Todd. Consequently, the titers recorded in this paper *are given in terms of Todd's standard serum*. Titers above 100 units per cc. are considered to be indicative of infection by hemolytic streptococci.

### *Results of antistreptolysin tests*

Repeated tests have confirmed the assertions of Hodge and Swift (18) that the combining power of streptolysin remains constant over a considerable period of time (provided the streptolysin has been properly prepared, reduced, and stored in the cold), while during the same period the hemolytic titer may vary appreciably. Their statement has been repeatedly confirmed that the antistreptolysin titer of serum remains stable when the serum is kept sterile and cold.

The specificity of the antistreptolysin test (5, 19) has been amply demonstrated by testing for their antistreptolysin content several sera from animals immunized to a variety of organisms. Practically without exception, the antistreptolysin titers of these immune sera were low (averaging 95 to 45 units or less per cc.). In addition, several human sera possessing high antistreptolysin titers were tested for staphylococcal antihemolysin. The low staphylococcal antihemolysin titers which were obtained confirms Todd's assertion that the hemolysins of streptococci and staphylococci are not serologically related (18).

*Rheumatoid arthritis.* Antistreptolysin determinations were done on 51 sera from 45 patients. The titers obtained are recorded in Table IV. Eighteen of these sera (representing 15 persons) gave titers definitely above the normal range (over 100 units per cc.) and the titers of the remaining 33 sera were within the normal range (100 units or less per cc.). It is interesting to note that, of the 18 sera giving high antistreptolysin titers, 17 also caused agglutination of either AB66 or Q33, or both, in significant titers. On the other hand, 29 sera which gave significant agglutination titers with these two streptococci possessed antistreptolysin titers within the normal range. Of five sera which gave little or no agglutination with AB66 or Q33, four also possessed normal antistreptolysin titers.

*"Control" groups.* Antistreptolysin determinations were done on 68 sera from 56 cases other

TABLE IV  
*Antistreptolysin titers*

Serial number of serum	Units per cc.	Approximate equivalent in fraction of cc.	Total number of sera giving titers indicated	Units per cc.	Approximate equivalent in fraction of cc.
Proven infections by hemolytic streptococci			Rheumatoid arthritis		
		cc.			cc.
1	95	0.0105	5	<47	0.0350
2	285	0.0035	13	47	0.0212
3 (1)	285	0.0035	15	95	0.0105
(2)	437	0.0023	1	133	0.0075
4 (1)	475	0.0021	9	190	0.0052
(2)	952	0.0010	1	210	0.0047
5 (1)	1146	0.0008	1	285	0.0035
(2)	95	0.0105	4	380	0.0026
6	380	0.0026	1	571	0.0017
7 (1)	665	0.0015	1	952	0.0010
(2)	571	0.0017			
Rheumatic fever			Chronic arthritides other than rheumatoid arthritis		
8	47	0.0212	3	47	0.0212
9	95	0.0105	8	95	0.0105
10	95	0.0105	1	114	0.0087
11	190	0.0052	1	190	0.0052
12	190	0.0052			
13	266	0.0037	Miscellaneous orthopedic conditions		
14	266	0.0037	1	47	0.0212
15	285	0.0035	1	57	0.0175
16	285	0.0035	1	95	0.0105
17	285	0.0035			
18	285	0.0035	Osteomyelitis		
19	285	0.0035	1	47	0.0212
20	342	0.0029	1	95	0.0105
21	761	0.0013	1	195	0.0051
22	1332	0.0007	3	285	0.0035
23 (1)	285	0.0035	1	380	0.0035
(2)	437	0.0023			
24 (1)	285	0.0035	Miscellaneous medical and surgical conditions		
(2)	285	0.0035	4	47	0.0212
(3)	190	0.0052			
25 (1)	380	0.0026	Normal persons		
(2)	475	0.0021	4	47	0.0212
(3)	761	0.0013	1	95	0.0105
(4)	665	0.0015			
(5)	475	0.0021			

than rheumatoid arthritis, representing a variety of orthopedic and medical conditions, and normal persons. Included in the control groups are 11 sera from 7 cases of proven infection by hemolytic streptococci; 25 sera from 18 cases of rheumatic fever; 23 sera from 22 miscellaneous orthopedic conditions (including 7 sera from 7 cases of osteomyelitis); 4 sera from 4 miscellaneous medical

or surgical conditions; and 5 sera from 5 healthy persons, with no history of preceding infection by hemolytic streptococci. The antistreptolysin titers of these sera are recorded in Table IV.

*Proven infections by hemolytic streptococci.* Sera were obtained from five patients with acute mastoiditis, one patient with infected varicose ulcer, and one patient convalescing from scarlet

the reciprocal of that dilution which just prevents hemolysis, as described above.

### *Standards*

In tests of this type it is obvious that standards should be established for the titration of streptolysin and antistreptolysin, so that the results obtained in various laboratories may all be recorded in similar terms. Only by this means may adequate comparisons be made of published reports from different laboratories. Recognizing this fact, Todd (19) has set aside a quantity of serum of known antistreptolysin potency, to be used as an arbitrary standard in antistreptolysin determinations.

The streptolysin of Hodge and Swift was standardized "with a number of sera furnished by Dr. Coburn . . . duplicates of these sera had been tested by Todd" (18). Through the courtesy of Dr. Hodge, samples of serum and of standardized streptolysin were obtained, and against these our own streptolysin and a few sera were standardized in a preliminary series of titrations. Recently, Dr. Todd kindly sent us some of his standard antistreptolysin serum which was used in a series of comparative tests with sera whose original titrations refer back to the standards supplied by Dr. Hodge. Thus checks on our streptolysin were obtained directly against Todd's standard serum, and indirectly against his standard, by means of serum supplied by Dr. Hodge.

*Antistreptolysin titer of normal serum.* In order to evaluate the results of antistreptolysin determinations, it is essential to know to what extent antistreptolysin is present in the serum of normal individuals, and to establish a normal value.

Todd (10), and Coburn and Pauli (11) have demonstrated that the maximum antistreptolysin titer of the serum of normal individuals is about 100 units per cubic centimeter. Parallel tests with Todd's serum and with sera which were titrated by the method of Hodge and Swift have shown that the value which we obtained for normal individuals is essentially equivalent to the value obtained by Todd. It would appear that, for the sake of exact comparison with other published results, the titers of antisera should be expressed in terms of some arbitrary standard, such as is represented by the serum which has been made

available by Todd. Consequently, the titers recorded in this paper *are given in terms of Todd's standard serum*. Titers above 100 units per cc. are considered to be indicative of infection by hemolytic streptococci.

### *Results of antistreptolysin tests*

Repeated tests have confirmed the assertions of Hodge and Swift (18) that the combining power of streptolysin remains constant over a considerable period of time (provided the streptolysin has been properly prepared, reduced, and stored in the cold), while during the same period the hemolytic titer may vary appreciably. Their statement has been repeatedly confirmed that the antistreptolysin titer of serum remains stable when the serum is kept sterile and cold.

The specificity of the antistreptolysin test (5, 19) has been amply demonstrated by testing for their antistreptolysin content several sera from animals immunized to a variety of organisms. Practically without exception, the antistreptolysin titers of these immune sera were low (averaging 95 to 45 units or less per cc.). In addition, several human sera possessing high antistreptolysin titers were tested for staphylococcal antihemolysin. The low staphylococcal antihemolysin titers which were obtained confirms Todd's assertion that the hemolysins of streptococci and staphylococci are not serologically related (18).

*Rheumatoid arthritis.* Antistreptolysin determinations were done on 51 sera from 45 patients. The titers obtained are recorded in Table IV. Eighteen of these sera (representing 15 persons) gave titers definitely above the normal range (over 100 units per cc.) and the titers of the remaining 33 sera were within the normal range (100 units or less per cc.). It is interesting to note that, of the 18 sera giving high antistreptolysin titers, 17 also caused agglutination of either AB66 or Q33, or both, in significant titers. On the other hand, 29 sera which gave significant agglutination titers with these two streptococci possessed antistreptolysin titers within the normal range. Of five sera which gave little or no agglutination with AB66 or Q33, four also possessed normal antistreptolysin titers.

*"Control" groups.* Antistreptolysin determinations were done on 68 sera from 56 cases other

TABLE IV  
*Antistreptolysin titers*

Serial number of serum	Units per cc.	Approximate equivalent in fraction of cc.	Total number of sera giving titers indicated	Units per cc.	Approximate equivalent in fraction of cc.
Proven infections by hemolytic streptococci			Rheumatoid arthritis		
1	95	0.0105	5	<47	0.0350
2	285	0.0035	13	47	0.0212
3 (1)	285	0.0035	15	95	0.0105
(2)	437	0.0023	1	133	0.0075
4 (1)	475	0.0021	9	190	0.0052
(2)	952	0.0010	1	210	0.0047
5 (1)	1146	0.0008	1	285	0.0035
(2)	95	0.0105	4	380	0.0026
6	380	0.0026	1	571	0.0017
7 (1)	665	0.0015	1	952	0.0010
(2)	571	0.0017	Chronic arthritides other than rheumatoid arthritis		
Rheumatic fever			3	47	0.0212
8	47	0.0212	8	95	0.0105
9	95	0.0105	1	114	0.0087
10	95	0.0105	1	190	0.0052
11	190	0.0052	Miscellaneous orthopedic conditions		
12	190	0.0052	1	47	0.0212
13	266	0.0037	1	57	0.0175
14	266	0.0037	1	95	0.0105
15	285	0.0035	Osteomyelitis		
16	285	0.0035	1	47	0.0212
17	285	0.0035	1	95	0.0105
18	285	0.0035	1	195	0.0051
19	285	0.0035	3	285	0.0035
20	342	0.0029	1	380	0.0035
21	761	0.0013	Miscellaneous medical and surgical conditions		
22	1332	0.0007	4	47	0.0212
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(3)	190	0.0052			
25 (1)	380	0.0026			
(2)	475	0.0021			
(3)	761	0.0013			
(4)	665	0.0015			
(5)	475	0.0021			

than rheumatoid arthritis, representing a variety of orthopedic and medical conditions, and normal persons. Included in the control groups are 11 sera from 7 cases of proven infection by hemolytic streptococci; 25 sera from 18 cases of rheumatic fever; 23 sera from 22 miscellaneous orthopedic conditions (including 7 sera from 7 cases of osteomyelitis); 4 sera from 4 miscellaneous medical

or surgical conditions; and 5 sera from 5 healthy persons, with no history of preceding infection by hemolytic streptococci. The antistreptolysin titers of these sera are recorded in Table IV.

*Proven infections by hemolytic streptococci.* Sera were obtained from five patients with acute mastoiditis, one patient with infected varicose ulcer, and one patient convalescing from scarlet



fever. The first specimens of serum were obtained from all but one of the patients from one and one-half to eight weeks after the onset of infection. Hemolytic streptococci were isolated in every instance.

It is obvious that the titers obtained are considerably above the normal range and are confirmatory of the proven infection by hemolytic streptococci. In one case of mastoiditis, an originally high titer of 1146 units (20 days after mastoidectomy) was followed six months later by a normal titer of 95 units, when the patient had clinically recovered from the infection. The one serum from a case of mastoiditis which had a normal titer was obtained only four days after the onset of infection.

*Rheumatic fever.* Twenty-five sera from 18 patients with rheumatic fever were tested. The three sera which gave normal titers (100 units per cc. or less) were obtained from three patients at a time of inactivity of the disease. All three were in the hospital because of cardiovascular disease which followed an earlier acute rheumatic fever. None gave a history of an acute infection of the throat prior to the time of the antistreptolysin test. The titers of 22 sera from the 15 other patients were decidedly higher than normal. All of these patients had acute rheumatic fever, and all gave histories of acute infections of the upper respiratory tract just prior to the onset of the attack. The gradual rise in titer after an attack of acute rheumatic fever, and the subsequent fall in titer, coincident with clinical improvement are seen in three of the cases.

*Miscellaneous other conditions.* Of the 32 miscellaneous sera tested for antistreptolysin, only seven gave titers above normal. Two were from cases of spondylitis of the Marie-Struempell type. The other five were from cases of chronic osteomyelitis. The opportunity for secondary infection by streptococci in this type of case is obvious. The titers of five sera from normal healthy persons, with no history of immediately preceding infection by hemolytic streptococci, were well within the normal range.

#### COMMENT

The appearance of antistreptolysin in cases of proven hemolytic streptococcus etiology and in

such diseases as rheumatic fever, scarlet fever, and erysipelas (whose streptococcal etiology is generally accepted) seems to follow a recent infection, or an acute exacerbation of an older process. Courn and Pauli (11) have shown that in rheumatic fever antistreptolysin rises to a high titer soon after the onset of infection or a recrudescence of the disease, and drops with a return to the quiescent state.

The presence of antistreptolysin in titers above normal in some cases of rheumatoid arthritis is interesting and suggestive, in view of the present general tendency to assume some relationship of streptococci to this disease. The fact that rheumatoid arthritis is a chronic disease obviously precludes the possibility of obtaining a high percentage of antistreptolysin titers above the normal range. However, it may be pointed out that, with one exception, the sera from cases of rheumatoid arthritis which possessed high antistreptolysin titers also caused agglutination of hemolytic streptococci in significant titer. When positive in high titer, antistreptolysin determinations may serve as additional evidence of the recent association of hemolytic streptococci with rheumatoid arthritis.

#### SUMMARY

Agglutinins for hemolytic streptococci in high titer were demonstrated in the sera of a majority of patients (85 per cent) with rheumatoid arthritis. Agglutination of hemolytic streptococci in high titer by a large percentage of sera was not obtained in other chronic arthritides.

No correlation was found between the agglutination titer and the age of the patient, duration of the disease, number of joints involved, or sedimentation rate, in cases of rheumatoid arthritis.

Antistreptolysin was present in titers above the normal range in the sera of patients with proven infections by hemolytic streptococci, and with acute rheumatic fever. There was a tendency for the antistreptolysin titer to return to within the normal range some time after convalescence.

Antistreptolysin in titers definitely above normal were found in about one-third of the sera from patients with rheumatoid arthritis. With one exception, these high titers accompanied high agglutination titers.

The presence of agglutinins for hemolytic strep-

tococci in sera from patients with rheumatoid arthritis is suggestive of an association of these organisms with this disease. Additional suggestive evidence may be offered by the presence of antistreptolysin, when it is found in titers above normal.

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# SULFUR METABOLISM IN CYSTINURIA<sup>1</sup>

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Although the literature contains numerous studies of cystinuric cases, further data on the metabolism of various sulfur compounds in the cystinuric individual is desirable, not only for the light it may throw on an interesting anomaly but also because the cystinuric may be valuable for the investigation of the course of sulfur metabolism in the normal individual. Among all the elements concerned in animal metabolism, sulfur is unique in that it undergoes the largest change of valence, the maximum valence change of  $-2$  to  $+6$  being accomplished when hydrogen sulfide is oxidized to sulfate, and a change only slightly smaller being involved in the normal metabolism of cystine. Since the magnitude of this change is not paralleled by any other element it hardly seems surprising that some individuals fail to accomplish complete oxidation of ingested sulfur. Indeed we might reasonably expect cystinuria to be of much more common occurrence than it is. The following report deals with the results of administering certain sulfur compounds to a cystinuric.

## EXPERIMENTAL

The subject, a 14 year old boy, had a history of kidney involvement with a right nephrectomy for multiple kidney stones at age 12. The surgical aspects of the case have been commented on recently by Herman and Lee (1). The subject is Case II in their report. Cystine has been constantly present in the subject's urine, quantitative determinations showing excretion of 0.4 to 0.5 gram cystine per day. However, examination of the urines of both parents gave negative results. At the time of our experiments the subject weighed 35 kilos and was otherwise in normal physical condition.

<sup>1</sup> A preliminary abstract of this paper was presented at the Pittsburgh meeting of the American Association for the Advancement of Science, December 1934.

A preliminary abstract of the work was published in the Proceedings of the Society of Experimental Biology and Medicine 1934, 32, 455.

Total sulfur was determined by the Benedict-Denis procedure and total sulfate-sulfur after hydrolysis with HCl. In all cases the determination was made gravimetrically as BaSO<sub>4</sub>. Cystine was determined by the Sullivan colorimetric procedure as modified by Brand *et al.* (2).

Table I shows the sulfur distribution in 24-hour urine samples of the subject at various periods over several months. With the exception of the experiments of September 23 and September 24, the subject was on normal miscellaneous diet with a somewhat higher sulfur intake than would be furnished by the two quarts of milk ingested per day (and for one day previous) during the experiment of September 23 and 24. On May 24, ingestion of 10 grams sodium bicarbonate daily was begun. Although direct cystine determinations were not made during the April period, they were made for some days before May 24 (not recorded on the table) and the results indicate that the ingestion of sodium bicarbonate has no effect on the degree of sulfur oxidation nor on the cystine output. In this we are in agreement with the results of Lewis and Lough (3) and Robson (4), as opposed to those of Looney, Berglund and Graves (5). The lack of effect of either sodium bicarbonate or sodium citrate in decreasing the cystine output of the cystinuric has been confirmed by later experiments. During the latter part of May, the sodium bicarbonate was replaced by an equivalent amount of sodium citrate which was ingested daily until the September experiments. No effect on the cystine output or on the sulfur distribution was observed. We feel, therefore, that the only object to be gained by the feeding of base to the cystinuric patient is that of keeping the cystine more completely in solution and of preventing formation of calculi. In this, we are merely increasing the natural tendency of cystinuric urines to be more alkaline than those of normal subjects because of the usual restriction of protein in the diet and also because of the ex-

TABLE I  
Oxidation of sulfur by a cystinuric subject

Date	Vol- ume	Nitro- gen N	Total S	Sulfate S	Unoxi- dized S	Percen- tage ox- idation	Cys- tine S	Cystine S per cent of unoxi- dized S	Remarks
	cc.	grams	grams	grams	grams	per cent	grams	per cent	
April 23	3400	7.58	0.632	0.357	0.275	56.5			Normal hospital diet
April 24	3700	7.30	0.589	0.370	0.219	62.8			" " "
April 25	3100	6.22	0.614	0.330	0.284	53.8			" " "
April 26	2670	6.80	0.542	0.356	0.186	65.7			" " "
April 27	1810	7.02	0.669	0.423	0.246	63.2			" " "
May 24	1320	6.82	0.604	0.398	0.206	65.9	0.099	48.0	10 grams NaHCO <sub>3</sub> per day
May 25	1760	6.86	0.566	0.332	0.234	58.7	0.134	57.2	" " " "
May 26	1300	7.06	0.551	0.314	0.237	57.0	0.107	45.1	" " " "
May 27	1280	8.02	0.583	0.368	0.215	63.1	0.112	52.1	5 grams glutamic acid + 2.55 grams gly- cine daily in addition to NaHCO <sub>3</sub>
May 28	780	5.83	0.456	0.269	0.187	59.0	0.087	46.5	
May 30	980	7.19	0.533	0.346	0.187	64.9	0.093	49.7	
May 31	1060	6.90	0.485	0.305	0.180	62.9	0.105	58.3	
June 1	950	6.69	0.496	0.302	0.194	60.9	0.108	55.7	
September 23	1245	6.77	0.434	0.225	0.209	51.8	0.100	47.8	Milk (2 quarts) + crackers and fruit
September 24	800	7.28	0.471	0.274	0.197	58.2	0.080	40.6	" " " " " "
October 27	1230	7.78	0.679	0.422	0.257	62.2	0.094	36.7	Egg diet (55 grams egg protein)
October 28	740	8.13	0.902	0.607	0.295	67.3	0.078	26.3	" " " " " "
November 5	1140	4.88	0.528	0.268	0.260	50.7	0.080	30.8	Low protein (30 grams protein)
November 6	960	4.60	0.513	0.257	0.256	50.1	0.081	31.5	" " " " " "
September 23	715	5.87	0.684	0.567	0.117	82.9			Normal subject-same age. Milk (2 quarts) + crackers and fruit
September 24	1065	9.15	0.711	0.590	0.121	83.0			

cretion of an appreciable portion of the sulfur as cystine instead of as sulfuric acid. The fact that in cystinuria one of the principal sources of urinary acidity (sulfuric acid) is diminished in quantity furnishes, to a partial degree, the alkalinity favorable to keeping the cystine in solution. If we compare the sulfur oxidation in normal with that in cystinuric subjects and calculate the effect of this deficiency in sulfuric acid in the cystinuric on the primary-secondary phosphate ratio (the chief buffer system of normal urine), we find that the cystinuric urine should average from 0.4 to 0.8 pH higher than the normal depending on variations in sulfur and phosphate output. The urinary acidity of the present cystinuric has been compared with that of a normal subject of the same age and sex, both being on identical diets for several days. Complete urine samples collected in each case over a period of two and one-half days gave average pH values of 6.5 for the normal and 7.1 for the cystinuric.

The solubility curve of cystine with change in pH demonstrates the possibility of increasing its

solubility at higher pH values and makes it difficult to evaluate the conclusions of Patch (6) as to the lack of efficacy of alkali. In the case of our present subject, x-ray examination has indicated a definite improvement resulting from administration of alkali. It is obvious that if a subject has already deposited small calculi or "gravel," administration of sufficient alkali to markedly increase the solubility of the cystine and cause it to be excreted in solution will produce a temporary rise in the total cystine excretion.

The suggestion has sometimes been advanced that the metabolism of cystine is so bound up with the formation of glutathione that administration of glycine and glutamic acid might decrease cystine excretion. While there is little to support such a view we thought it worth while to feed these two amino acids in equimolar amounts for a period of some days. As indicated in Table I, 5.0 grams glutamic acid + 2.55 grams glycine (supplying a total of 0.95 gram nitrogen) were fed daily for approximately one week. Neither during that period nor for some days following

was there any noticeable effect on the amount of cystine excreted. In this, our results are as completely negative as those of Robson (4) when glutamic acid alone was fed.

In investigating the metabolism of sulfur compounds administered by mouth we have also used a procedure in which urine collections were made by two hour periods during the day and for the following 12 hour period overnight. The subject, having been kept on a nonprotein diet the day before the experiment, voided and discarded his urine at 6 a.m. Samples were then collected at 7, 9, and 11 a.m., etc. until 7 p.m. The following 12 hour period (7 p.m. to 7 a.m.) was also collected. Since the compound to be studied was ingested at 7 a.m. on the day of the experiment, 24 hours were allowed for its elimination, a period insufficient in most cases, for complete elimination of the sulfur. On the day of the experiment no food was ingested until the evening meal and practically no protein was allowed until the following day. Constant quantities of water were taken hourly but in spite of this the bi-hourly output often varied considerably. The sulfur compound was ingested by mouth in quantity amounting to 0.40 gram sulfur.

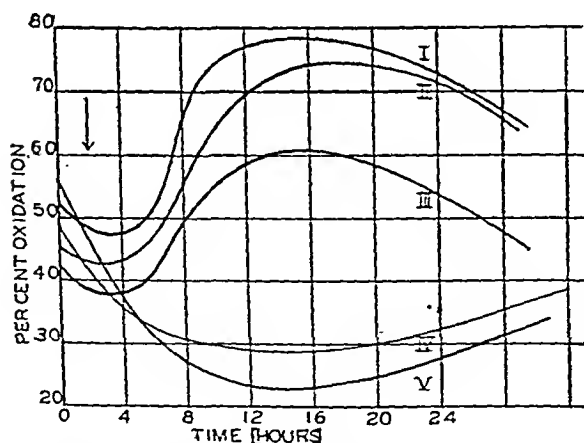


FIG. 1. PERCENTAGE OXIDATION OF SULFUR RESULTING FROM ADMINISTRATION OF VARIOUS SULFUR COMPOUNDS IN AMOUNTS EQUIVALENT TO 0.4 GRAM SULFUR.

- I = l-cystine
- II = dl-cystine
- III = dl-methionine
- IV = blank
- V = cysteic acid

Arrow indicates time of administration of compound

Since the chief variation in the effects of different sulfur compounds is to be found in the proportion of the sulfur oxidized and excreted as sulfate, the results of a number of such experiments are summarized in Figure 1 by plotting percentage oxidation against time in hours. The percentage oxidation represents the ratio of total sulfate sulfur to total sulfur in the urine specimen. This percentage is higher in the samples collected earlier in the day but as the experiment more nearly approaches a fasting basis, this figure in blank experiments becomes approximately 30 per cent. Under the subject's normal dietary conditions this percentage is roughly doubled. The later rise in the curve is the result of the resumption of food intake on the sulfur partition in the sample collected the following morning.

In agreement with the classical picture of cystinuria, the present subject is able to oxidize free cystine, administered by mouth, as well as a normal individual. This is indicated by the rise in the curves for both l- and dl-cystine during the early part of the 24 hour period.

The comparative feeding of l- and racemic cystine was prompted by the remarkable results reported by Loewy and Neuberg (7) who found a cystinuric subject capable of oxidizing "stone cystine" to sulfate but incapable of so oxidizing "protein cystine" (from hair). The suspicion that such results might be caused by difference in optical configuration (the "protein cystine" having been racemized during preparation) is increased by the findings of duVigneaud, Craft and Loring (8). These investigators obtained, with rabbits, a percentage oxidation for d-cystine of approximately half the magnitude of that for the l-isomer. While it is true that our figures show somewhat slower oxidation of racemic cystine during the first 12 hours, the differences are not great and the results, as a whole, more nearly approach those of Hele and Pirie (9) on dogs and Lawrie (10) on rats. The figures for the excess sulfate excreted as a result of cystine ingestion, when corrected for the average fasting level, indicate a somewhat higher recovery of the sulfur for l- than for racemic cystine, but here again the differences are not highly significant, considering the difficulty of assigning a definite value for the average fasting level.

In the case of the l-cystine experiment, about 75 to 80 per cent of the sulfur administered, was recovered as sulfate-sulfur in 24 hours, whereas the same calculation applied to the experiment with racemic cystine indicates a recovery of 60 to 65 per cent. In neither case was any significant rise in unoxidized sulfur noted. In studying the absorption of cystine from dogs with isolated intestinal loops, Andrews and Johnston (11) obtained results indicating slightly more rapid absorption of racemic cystine. This, however, by no means implies a more rapid or complete metabolic oxidation.

Although administration of cysteic acid to the cystinuric individual would not be expected to produce results different from those of the normal, such an experiment appears not to be recorded in the literature and we therefore included in Figure 1, the curve of one of several such experiments, conducted in the same way as described above. Since the cysteic acid is not converted to sulfate by the organism, the output of sulfate-sulfur remains relatively constant while the unoxidized portion increases moderately and the oxidation percentage drops. In another cysteic acid experiment, the percentage of sulfur oxidation reached the low level of 17.4 per cent. However, the absolute increase in sulfur elimination was in

all cysteic acid experiments so slight that the sulfur recovery amounted to only 30 to 40 per cent of that fed. This is all the more surprising in view of the very rapid absorption of cysteic acid from intestinal loops of dogs observed by Andrews and Johnston (11), but accords with the low recoveries of sulfur observed by Schmidt and Clark (12) after feeding cysteic acid to dogs. Further investigation of the cause of these low recoveries of cysteic acid, both in the normal and the cystinuric organism is in progress.

The administration of methionine to the cystinuric individual presents several features of much interest. Not the least of these is the suggestion of Brand, Cahill and Harris (13) that cystinuria may be essentially a disturbance in methionine metabolism as indicated by their finding that the output of cystine by a cystinuric individual was much increased (nearly doubled) by administration of large doses of methionine. The methionine curve in Figure 1, obtained by feeding an amount of methionine equivalent to 0.40 gram sulfur, demonstrates that this amino acid is not nearly so readily oxidized to sulfate as is cystine.

In Table II are presented the results of a longer experiment in which methionine equivalent to 0.50 gram sulfur was fed after a preliminary period, and twelve hour urine samples were collected for

TABLE II  
*Administration of dl-methionine*  
(Urine samples collected in five 12-hour periods)

Period	Volume	Total N	Total S	Sulfate S	Unoxidized S	Percentage oxidation	Cystine S	Methionine S	$\alpha$ X cc. (2 dm.)
	cc.	grams	grams	grams	grams		grams	grams	degrees
<i>Cystinuric subject</i>									
I	640	4.63	0.294	0.180	0.114	61.2	0.058	0.014	-115
II*	690	3.92	0.453	0.243	0.210	53.6	0.062	0.084	-138
III	350	4.23	0.364	0.249	0.115	68.4	0.052	0.020	-70
IV	490	3.72	0.276	0.152	0.124	55.1	0.042	0.018	-113
V	710	4.19	0.310	0.173	0.137	55.8	0.071	0.017	-135
<i>Normal subject</i>									
I	350	5.34	0.383	0.327	0.056	85.4		0.019	-39
II*	1160	6.83	0.772	0.633	0.139	82.0		0.069	-116
III	560	6.42	0.457	0.393	0.064	86.0		0.028	-45
IV	800	6.28	0.511	0.440	0.071	86.1		0.023	-64
V	310	4.10	0.262	0.211	0.051	80.5		0.014	-43

\* 2.33 grams. dl-methionine (=0.50 gram S) administered at the beginning of Period II.

48 hours after its administration. In this case, the cystinuric was compared with a normal subject of the same age and sex. Both subjects were kept on the standard milk-cracker diet used for the experiments of September 23 and September 24 in Table I during the day preceding the first period of urine collection as well as during the entire experiment. The methionine was administered by mouth at the beginning of Period II.

Examination of Table II shows that here again, administration of methionine has little influence on the percentage oxidation of the sulfur with either subject since it produces proportional increases in both oxidized and unoxidized fractions. The figures for cystine sulfur excreted by the cystinuric demonstrate that this increase is not to be accounted for by an increased cystine output. The sharp rise in unoxidized sulfur noted in Period II is not accompanied by a significant rise in cystine sulfur while the rise in the latter in Period V is evidently the result of an increased urine volume at that time (see below). One striking difference to be noted between the two subjects is the promptness with which the normal subject excreted the extra sulfur administered as contrasted with the slower and somewhat erratic excretion by the cystinuric patient.

The methionine administered was the *dl*-form whereas the natural isomer is the *l* form ( $[\alpha]_D = -7.2$ ). On the supposition that the organism most readily oxidizes the natural isomer and discards all or part of the *d*-form, we have sought to account for the fact that *dl*-methionine is less completely oxidized than cystine and gives rise to increases in the unoxidized S fraction. For this reason methionine was determined directly in these urines.

The figures were obtained by the method of Baernstein (14). For each determination a 50 cc. sample of the urine was evaporated to dryness below the boiling point and the determination was made on this dried residue. It was demonstrated that methionine, added to the urine before evaporation, could be 94 to 96 per cent recovered. Normal urines give a small titration by this method, which, expressed in terms of methionine sulfur, amounts to about 0.02 to 0.04 gram per 24 hours. The table shows that both normal and cystinuric subjects exhibited 4 to 6 fold increases

in this value during the period immediately following methionine ingestion. However, the absolute amount of the increase accounts for not more than 10 to 15 per cent of the methionine sulfur ingested although it does account for 60 to 70 per cent of the increase observed in the unoxidized sulfur fraction. The fact that the methionine was in the *dl* form lends some support to the view that in these experiments oxidation may be less complete than would have been the case had the naturally occurring isomer been administered.

The last column of the table shows the product obtained by multiplying the optical activity observed with D-light in a 2 dm. tube at 25° C. by the volume of the sample for that period. The resulting figure is therefore an arbitrary measure of the excretion of some levorotatory constituents and is obviously higher with the cystinuric because of the presence of *l*-cystine. With moderately constant urinary volumes, marked increases in cystine output should be evident in these figures. However, the only case in which such an increase occurs is in Period II of the normal subject when the methionine ingestion was followed by marked diuresis. That this increase in the optical figure is not due to excretion of cystine or homocystine is evidenced by the fact that all samples from the normal subject gave negative cyanide-nitroprusside tests. In this, we fail to confirm the results of Virtue and Lewis (15) and Vars (16) with rabbits and dogs respectively but it should be noted that our dose, per kilo of body weight, is far less than that used by them. The possibility of formation of homocystine from the methionine in the cystinuric is practically excluded by the fact that Folin-Marenzi determinations (which respond to both cystine and homocystine) have given values for cystine which were not appreciably higher than those obtained by the more specific Sullivan method.

In order to investigate the effect of still larger doses of methionine on the cystinuric organism, a similar experiment was run on the same subject over a period of fifteen days under carefully controlled dietary conditions. The diet used contained 300, 50 and 100 grams respectively of carbohydrate, protein, and fat daily. Twenty-four hour urine collections were made. After establishing this metabolic level for several days, the



subject was given a 5.0 gram dose of cystine in water suspension. On the 4th, 5th and 6th days after cystine ingestion large doses of dl-methionine were given. The results are summarized in Table III.<sup>2</sup>

The results of this experiment confirm our previous conclusions: that with the present cystinuric subject, no very marked increase in cystine

may therefore conclude that there is no evidence of excretion of homocystine. The figures for methionine sulfur again show an increase following methionine ingestion although the amount is small as compared with the amount of methionine ingested. The column showing optical activity (Table III) confirms the previous conclusion as to the constancy of cystine output.

TABLE III  
*Administration of di-methionine*  
(Twenty-four hour urine samples)

Day	Volume	N	Creatinine	Total S	Sulfate S	Unoxidized S	Percentage oxidation	Cystine S	Methionine S	$\alpha \times \text{cc.}$ (2 dm.)
	cc.	grams	grams	grams	grams	grams	per cent	grams	grams	degrees
1	1840	7.27	0.78	0.712	0.236	0.476	33.6	0.147	0.038	-320
2	1520	7.52	0.67	0.685	0.320	0.365	46.7	0.126	0.041	-340
3	1580	7.31	0.75	0.756	0.297	0.459	39.3	0.124	0.039	-420
4	2000	7.71	0.82	0.616	0.362	0.254	58.8	0.135	0.028	-360
5*	1790	7.93	0.76	1.440	1.126	0.314	78.2	0.091	0.022	-300
6	1680	7.11	0.58	0.425	0.192	0.233	45.3	0.088	0.021	-320
7	1740	7.02	0.51	0.452	0.236	0.216	52.2	0.090	0.025	-320
8	1540	6.58	0.81	0.518	0.267	0.251	51.5	0.087	0.022	-400
9†	1490	7.03	0.73	0.824	0.473	0.351	57.4	0.101	0.051	-280
10†	1620	6.92	0.68	0.788	0.461	0.327	58.5	0.102	0.044	-260
11‡	2000	7.51	0.51	0.952	0.645	0.307	67.7	0.108	0.077	-460
12	2000	7.16	0.66	0.496	0.238	0.258	48.0	0.111	0.021	-440
13	1960	7.01	0.69	0.576	0.313	0.263	54.3	0.108	0.019	-460
14	1760	6.83	0.52	0.404	0.217	0.187	53.7	0.099	0.023	-420
15	1480	6.87	0.80	0.572	0.276	0.296	48.3	0.095	0.020	-340

\* 5 grams l-cystine (=1.333 gram S) administered at the beginning of the 5th day.

† 2 grams dl-methionine (=0.43 gram S) administered at the beginning of both 9th and 10th days.

‡ 5 grams dl-methionine (=1.075 gram S) administered at the beginning of the 11th day.

excretion follows the ingestion of large amounts of methionine. It should also be noted that what increase in cystine output was observed accompanied the larger urine volumes. We have noted, on several other occasions, that diuresis is accompanied by an increased cystine output. The divergence between our results in this particular and those of Brand and coworkers (13), suggests that there exists considerable variation between cystinuric subjects, and points to the desirability of experimentation with as wide a range of subjects and conditions as possible. The figures for cystine sulfur were obtained, as before, by the Sullivan method but no appreciable increase was obtained by the use of the Folin procedure. We

The percentage recovery of the sulfur is, as would be expected, somewhat low. One could hardly expect to administer doses of cystine and methionine of the size used here without having considerable loss in the feces.

In spite of the constancy of the total nitrogen there is obviously considerable variation in the creatinine figures, and it is somewhat suggestive that the most marked variations date from the day on which the large dose of cystine was given. The variability of creatinine output in cystinuric subjects has been commented on both by Alsberg and Folin (17) and by Brand, Harris and Biloon (2). These authors ascribe a rise in creatinine which they observed to an unusual amount of exercise on the part of their subjects and since our present subject was leading a normal school life during the experiment with the daily amount of exercise varying considerably this may account for our variations. The extremely cooperative attitude of the subject as well as the constancy of the

<sup>2</sup> We wish to acknowledge the kindness of Dr. Erwin Brand of the New York State Psychiatric Institute in furnishing the dl-methionine used in this experiment. We wish also to acknowledge the assistance of Kathleen C. Andrews in controlling the dietary conditions of the experiment and in carrying out numerous analyses.

total nitrogen figures negates any assumption of incomplete collections.

TABLE IV

*Increase in free cystine content of cystinuric urines on standing*

Free cystine content of sample		
At once	After 10 days	After 100 days
grams	grams	grams
0.034	0.044	0.044
0.023	0.036	
0.050	0.072	
0.034	0.043	0.044
0.036	0.052	
0.030	0.038	0.036
0.056	0.056	0.041
0.039	0.038	0.037
0.050	0.057	0.061
0.039	0.041	

Table IV illustrates an interesting quality exhibited by some cystinuric urines. Brand, Harris and Biloon (2) have pointed out that the Sullivan colorimetric method for cystine when applied to very freshly voided cystinuric urines, sometimes gives values which are decidedly lower than those obtained when the same method is applied later to the same samples. Because of the well known specificity of the Sullivan procedure and its usual failure to respond to cystine complexes, this increase was explained by assuming the excretion of an unstable complex which quickly decomposed. The figures recorded in Table IV show that in most cases we substantiate these findings as far as our subject is concerned. It will be noted that this increase, when it is observed, is maintained on standing for over 3 months (at 0° and preserved with chloroform). We have also noted that cystinuric urines over eight months old show practically no further change in cystine concentration. The statement made by Magnus-Levy (18) that such urines lose their cystine after some months is hard to explain except on the assumption that slow racemization occurred and that the higher solubility of the racemized cystine adversely affected the precipitation method he used.

The hypothetical cystine complex is, however, far less stable than the data in Table IV imply. We have observed an increase of practically the same magnitude after keeping the fresh sample for 24 hours at 0° C. The instability of the com-

plex is further emphasized by the fact that our present subject, while giving evidence of its excretion, has also formed deposits of practically pure cystine. As far as we are aware, no cystinuric urine on record has ever given a *negative* Sullivan test when freshly voided, and certainly such a finding could hardly be expected in any subject with even small deposits of free cystine in kidney or bladder.

Further investigation of the chemistry of this complex is highly desirable since some means of increasing its stability might have practical bearing on the treatment of cystinuria; the excretion of a soluble complex might avoid the dangers of calculus formation. At present, however, we must admit the possibility that this increase in color may be merely due to the influence of some other urinary constituents on the Sullivan reaction.

#### SUMMARY

A case of cystinuria accompanied by calculus formation has been investigated with regard to the metabolism of various sulfur compounds by the subject.

The cystine output is unchanged by daily administration of alkali (sodium bicarbonate or sodium citrate) over a period of several months. Deposition of calculi has, however, evidently been prevented by this means.

Administration of equivalent amounts of glycine and glutamic acid is without effect on the rate of cystine excretion.

Administration of l-cystine by mouth results in practically complete oxidation of the latter; administration of dl-cystine is followed by slightly less efficient oxidation.

Cystic acid, administered by mouth causes no increase in sulfate excretion.

Following administration of dl-methionine to the cystinuric subject we have observed: (1) No significant increase in cystine excretion, (2) no excretion of homocystine, (3) definite but very slight excretion of methionine.

The increase reported by Brand and coworkers (2) in the apparent cystine content of these urines on standing has also been observed by us.

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# OBSERVATIONS ON SODIUM CHLORIDE RESTRICTION AND UREA CLEARANCE IN RENAL INSUFFICIENCY<sup>1</sup>

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The azotemia which accompanies hypochloremia has been ascribed by some to transient renal insufficiency of a purely functional type (Haden and Guffey (1924), Mellinshoff (1934)) and in severe cases to grave anatomical changes in the kidney (Brown et al. (1923)). Others believe that renal function per se is essentially unaffected by hypochloremia (Blum, Grabar and Van Cau-laert (1929a, b)) and that urea is retained by the body in order that the osmotic pressure of the body fluids may remain unchanged despite chloride loss. Diminished urinary excretion of nitrogen and the resulting azotemia are ordinarily used to measure renal insufficiency with the inherent assumption that renal insufficiency produces nitrogen retention primarily. Hartmann and Darrow (1928) suggest, however, that even in nephritis urea may be retained, not primarily because of reduction in the power of the kidneys to excrete urea but secondarily to compensate for the low osmotic pressure of the body fluids resulting from loss of electrolytes.

Most patients in whom significant hypochloremia is discovered are suffering also from the other effects of vomiting—dehydration, diminished food intake and oliguria. Nitrogen might therefore accumulate in the blood simply because of oliguria (Peters (1932), Kerpel-Fronius and Butler (1935)) and the increased protein destruction of starvation and dehydration (Peters (1932), Meyer (1932), Morawitz and Schloss (1932)). The diminished excretion of phenolsulphonphthalein (Brown et al. (1923)) and of ferments (Mellinshoff (1934)) might also, according to this view, be produced by marked oliguria without primary change in the functioning power of the kidney per se.

Conventional urea clearances with brief periods of urine collection have not revealed any change

in renal function during chloride administration or deprivation. Leiter (1926) varied salt intake and concluded, from functional studies involving 1-hour urine collections, that salt intake had no effect on the excretion of urea or phenolsulphonphthalein. Cope (1933) observed in unselected cases of renal disease that neither the rapid ingestion of 65 grams of sodium chloride nor restriction to 1 gram per day influenced urea clearance when determined over the usual two successive one-hour periods recommended by Möller, McIntosh and Van Slyke (1928).

On the other hand, a prolonged salt-poor diet diminishes the urinary excretion of nonprotein nitrogen (Hatcher and Sollmann (1903), Schoenthal (1929)) and is associated with a slow rise of blood urea nitrogen too frequently to be explained as a coincidence. Rigorous salt restriction and slight or moderate fluid restriction elevated the blood urea nitrogen in 44 of 104 hypertensive cases having no previous clinical evidence of renal insufficiency (Allen and Sherrill (1922)). Under a similar regime 15 of 33 nephritics and 14 of 33 diabetics showed a rising blood urea nitrogen. Allen and Sherrill state that occasionally salt restriction must be made less rigid when blood urea nitrogen rises too rapidly, or too much. McLester (1922) in repeating the observations of Allen and Sherrill found, in 7 of 10 hypertensive patients, that blood urea nitrogen and creatinine rose during salt restriction.

The relation between sodium chloride restriction and kidney function is admittedly an extremely difficult problem owing to the many factors involved. While variations in sodium chloride intake have often seemed to affect the renal elimination of nitrogen there is considerable conflict in both data and interpretation. It appeared that studies carried out by a slightly different approach might yield additional information concerning possible changes in renal function during

<sup>1</sup> The cost of this investigation was in large part defrayed by the Commonwealth Fund.

the administration and restriction of sodium chloride.

The figures of McLester (1922) indicate that blood urea nitrogen may rise at the rate of 1 mgm. per 100 cc. per day when salt is restricted. Calculation shows that, if urea be distributed through 70 per cent of body weight, to increase the concentration of urea nitrogen by 1 mgm. per 100 cc. per day in a 70 kilo man would require the retention of 500 mgm. of urea nitrogen and a decrease of 24-hour excretion from a normal of 10 grams to 9.5 grams per day. If protein intake and metabolism remain constant, retention of urea nitrogen at this rate would indicate that urea clearance had been diminished by 5 per cent, e.g. from 100 to 95 per cent of normal. Urea nitrogen would be eliminated once more at the rate of 10 grams per day when the blood urea nitrogen became high enough to compensate for the reduced clearance. Such small changes in renal function will be difficult to detect by direct studies of urea clearance, though the cumulative effect of slightly decreased urea clearance might well produce in the course of days a significant rise in the concentration of blood urea nitrogen.

It is doubtful whether any practicable number of 2-hour urea clearances would reveal changes in renal function amounting even to 10 or 15 per cent because, as usually determined, urea clearance varies widely in the same individual. Bruger and Mosenthal (1932) state that urea clearance may vary as much as 100 per cent from hour to hour or from day to day in the same individual. This variability lessens as renal function diminishes in the course of disease, but successive determinations still show but little constancy. Thus Page (1934) records for single patients urea clearances which vary as much as 50 per cent even when two 1-hour clearances are averaged. When the clearances during single 1-hour periods are compared the variation is greater. Van Slyke, Alving and Rose (1932) report urea clearances ranging on the same day from 3.4 to 12.2 per cent of normal in a patient with terminal hemorrhagic nephritis.

It seemed possible that routine urea clearance determinations (Cope) and "urea concentration indices" (McLean) failed to reveal changes in renal function during salt deprivation because

spontaneous variations in renal function over brief periods were far greater than the relatively small changes that were to be measured. Therefore, in the studies described in this paper urea clearances were determined in 2 normal subjects during short (1-hour) and long (24-hour) periods of urine collection. Having found that prolonging the period of urine collection diminished to a certain extent the variability of the urea clearance figures, the method was applied to the study of changes in renal function during salt deprivation in three cases of renal insufficiency, one with hypochloremia and marked azotemia, two with normal plasma chlorides and trifling elevations of blood urea nitrogen.

While the observed changes in 24-hour urea clearance were small, the direction of change was similar in all 3 patients, the blood urea nitrogen tending to rise, the urea clearance tending to fall when the intake of sodium chloride was diminished. The observations are described not as conclusive evidence but as findings which suggest that this method of study applied to other cases with conspicuous hypochloremia may aid in reconciling certain divergent views now expressed in the literature.

#### METHODS

The patients were placed under special and continuous nursing care with rigid supervision of food and fluid intake. Each article of diet was weighed accurately and the amount returned was recorded, so that the dietary figures represent the amount actually eaten. Sodium chloride, when administered, was dissolved in the drinking water. Urine was collected with scrupulous care over periods of 12 or 24 hours from 8:00 to 8:00, any deviation being recorded to the nearest minute. If a urine specimen was contaminated by feces the whole 12- or 24-hour collection was discarded. Blood samples were taken before breakfast to obtain fasting concentrations. The urea nitrogen in blood plasma was determined by the method of Van Slyke and Cullen (1914). Urinary urea nitrogen was determined by the same method after ammonia had been removed with permittit. Eisenman's (1929) modification of the Van Slyke and Sendroy method was used for the estimation of chloride in plasma and urine. On determining plasma chlorides a mixture of 95 per cent oxygen

and 5 per cent carbon dioxide was passed through whole blood slowly under oil for about 20 minutes before the plasma was separated. Creatinine in plasma and urine was determined by the method of Folin as used by Holten and Rehberg (1931). In order to keep the concentration of creatinine sufficiently high for color comparison it was occasionally necessary to dilute the plasma only five times instead of the usual ten.

The specific gravity of urine was determined by means of a pycnometer containing 25 cc. The total protein of urine was estimated by the gravimetric method of Folin and Denis (1914). The precipitated urinary proteins were dried overnight in an oven at 105° C. before weighing.

*The range of variations in 1-hour and 24-hour urea clearances in two normal subjects*

It was the purpose of these preliminary observations to determine whether urea clearances determined over periods of 24 hours were significantly more constant than urea clearances determined over the conventional 1-hour periods.

The urine of 2 normal subjects was collected in 24-hour periods extending approximately from 8 a.m. of one day to 8 a.m. of the day following. The exact duration of each period was recorded

to the nearest minute. No attempt was made to modify or control diet, fluid intake, environmental temperature or activity. Each completed specimen of urine was well mixed and its volume and urea nitrogen content were recorded. Venous blood samples for determining plasma urea nitrogen were collected twice daily, before breakfast and after the evening meal.

The results of these observations are shown in Table I. The urea clearances for the 24-hour periods were calculated as per cent of normal according to the equations of Möller, McIntosh and Van Slyke (1928). In these studies on normal subjects the average rate of urine formation was always below 2.0 cc. per minute so that the calculated 24-hour urea clearances were all "standard clearances."

The calculation of such 24-hour urea clearances introduces no objections which cannot be raised against 2-hour clearances; the difference being one of degree alone. The Van Slyke corrections for "maximal" and "standard" clearances were used since the effect of diuresis per se on the efficiency of urea excretion was thus eliminated as far as possible. It may be mentioned that neglecting this correction and calculating urea clearances simply in terms of "cc. per minute" (i.e.

TABLE I  
*Urea clearances over 24-hour periods*

24-hour periods	Plasma urea nitrogen		Urine		Urea nitrogen elimination	24-hour urea clearance	
	Fasting	After evening meal	Rate of formation	Urea nitrogen		Using average plasma urea nitrogen	Using fasting plasma urea nitrogen only
	mgm. per cent	mgm. per cent	cc. per minute	mgm. per cent		per cent normal	per cent normal

A. Subject K. E., 7 days.

1	12.0	14.4	.99	704	10.0	98	108
2	12.7	14.2	.72	902	9.4	105	112
3	13.2	14.4	.79	761	8.7	91	95
4	12.0	14.6	.69	887	8.8	103	114
5	11.4	10.0	1.20	476	8.2	90	85
6	10.0	11.5	.83	762	9.1	119	128
7	10.0	15.0	.75	792	8.6	102	127

B. Subject E. M. L., 5 days.

1	10.4	12.2	.63	867	7.9	112	122
2	10.3	13.5	.85	846	10.4	121	140
3	11.8	14.8	.78	1174	13.2	144	163
4	12.0	17.1	1.02	893	13.1	114	139
5	15.0	15.0	1.03	887	13.2	111	111

analogous to maximal clearance) led to the same conclusion as the calculations of "standard" clearance. It is recognized also that if absolute urea clearances were to be determined many blood samples should be taken during each 24-hour period. For comparative studies in individual patients it seemed advisable to take fewer blood samples and to multiply the number of periods, since it is manifestly impossible to perform venipunctures at intervals of 1 or 2 hours day after day.

No correction was made for surface area since variations in given subjects were being studied,

when determined over 1-hour periods in the same subject. The latter figures were obtained in the course of studies on the effect of environmental temperature on urea clearance but are quite comparable to the 24-hour clearances in which environmental temperature also varied considerably. The conventional technique was followed; urine was collected over two successive 1-hour periods with a blood sample at the end of the first hour. In addition breakfast was omitted. In half the determinations the subject was semirecumbent, in the other half he was walking about the room.

The average values recorded in the first column

TABLE II

*Comparison of variation of 24-hour and 1-hour urea clearances*

	Average	Highest	Lowest	Maximum variation from average
	<i>per cent normal</i>	<i>per cent normal</i>	<i>per cent normal</i>	<i>per cent</i>
A. Subject K. E.				
Calculations based on 7 24-hour periods				
(a) from average plasma urea nitrogen.....	101	119	90	+18, -11 (29)
(b) from fasting plasma urea nitrogen.....	110	128	85	+16, -23 (39)
Calculations based on 8 1-hour periods.....	87	106	63	+22, -28 (50)
B. Subject E. M. L.				
Calculations based on 5 24-hour periods				
(a) from average plasma urea nitrogen.....	120	144	111	+20, -8 (28)
(b) from fasting plasma urea nitrogen.....	135	163	111	+21, -18 (39)
Calculations based on 12 1-hour periods.....	110	178	80	+62, -27 (89)

absolute values being of secondary interest. The clearances were, however, calculated in two ways —(a) using the average of night and morning plasma urea nitrogen and (b) using fasting plasma urea nitrogen only. Except for one day in each subject the plasma urea nitrogen was higher in the evening than in the morning. Therefore the absolute figures for 24-hour urea clearances were lower when calculations were based on both morning and evening plasma urea nitrogen than when calculations were based on fasting plasma urea nitrogen alone. On successive days there was no consistent relation between changes in 24-hour urea clearance and total urea nitrogen output.

Table II compares the variation in urea clearance when determined over 24-hour periods and

of Table II all fell within the limits of normal. For each subject the average 24-hour clearance was higher than the average 1-hour clearance; this difference is, however, of little significance since the two series were not carried out simultaneously. Attention is called particularly to the lessened variability of urea clearance figures when urine is collected over longer periods. Twenty-four hour urine collections with a single (fasting) blood urea nitrogen yielded urea clearances whose total variation was rather less (39 per cent compared to 50 and 89 per cent) than that of the conventional urea clearance.

To detect possible small changes in urea clearance it seemed advisable therefore to use 12- or 24-hour urine specimens (a) because normal varia-

tion is slightly less and (b) because the continuous collection of urine gives a more complete average picture of urea elimination than would isolated samples of urine collected over briefer periods. The absolute urea clearance figures obtained by these longer periods of urine collection cannot at present be compared directly with the average normal established by Möller, McIntosh and Van Slyke (1928) who used shorter periods of collection. This limitation, however, does not invalidate the use of this method for detecting changes in urea excretion by a given individual under different regimes.

#### *Sodium chloride administration and 24-hour urea clearance in patients with renal insufficiency*

Case 1, A. P., female aged 43, married, was admitted to the hospital first on May 23, 1934, primarily for arthritic symptoms. In the course of study hypertension (180 systolic, 100 diastolic) and retinal sclerosis were discovered. The blood urea nitrogen was 108 mgm. per 100 cc. and routine urine examinations showed abundant albumin, low specific gravity, few or no casts and many red cells. A concentration test and cast count performed when the blood urea nitrogen was lower yielded the following results:

Restriction of fluid for 24 hours (Addis) elevated the specific gravity of the urine to only 1.010. The 12-hour volume was 450 cc., the reaction acid. Cast excretion totalled 24,000 per 12 hours with 30 per cent each of hyaline, granular and epithelial casts and 10 per cent of failure casts. Erythrocyte excretion was 15.0 million per 12 hours, leukocyte and epithelial cell excretion 1.0 million per 12 hours. The plasma protein percentage was normal and proteinuria amounted to 0.56 gram per 12

hours. Phenolsulphonphthalein elimination was 7 per cent in the first hour and less than 5 per cent in the second hour. Several 2-hour urea clearances indicated from 3 to 12 per cent of normal function. Chloride elimination (reported in terms of NaCl) was 2.38 grams per day; the plasma contained 102 milliequivalents of chloride per liter.

Under a regime of moderately increased fluid intake and low protein diet with salt ad lib., the plasma urea nitrogen fell to 50.9 mgm. per 100 cc. and remained almost stationary. From June 4 to 10 (Table III) sodium chloride was administered in dosage of 10 grams per day while creatinine and urea clearances were followed in a preliminary way. The rate of urine formation varied between 1.01 and 1.77 cc. per minute; blood urea nitrogen fell from 50.9 to 34.6 mgm. per 100 cc., the 24-hour urea clearance changing simultaneously from 4.7 to 8.3 per cent of normal. Chloride excretion was conspicuously below chloride intake during the first few days but rose finally to 15.8 grams on the seventh day of salt administration. The plasma chloride level was not appreciably altered.

During the next 6 days no sodium chloride was given except that contained in the diet. Chloride elimination, plasma chloride concentration and the 24-hour urea clearance diminished. Urea clearance fell from over 7.0 to 3.0 per cent of normal while plasma urea nitrogen simultaneously rose from 34.6 to 40.1 mgm. per 100 cc. The concentration of creatinine in plasma was high enough (6.95 mgm. per 100 cc.) to allow determining reasonably accurate creatinine clearances. The average creatinine clearance during sodium chloride administration was 10.1 cc. per minute. When salt was not added to the diet the creatinine clearance averaged 7.3 cc. per minute. Plasma creatinine fell from 6.95 to 6.1 mgm. per 100 cc. during sodium chloride administration, rising to 7.3 mgm. per 100 cc. when dietary salt alone was given. The patient was discharged with a blood urea nitrogen of 38 mgm. per 100 cc. and was advised to follow a regime of

TABLE III

Case 1. Effect of sodium chloride administration on urea and creatinine clearances with only partially controlled diet and moderate fluid intake

Date	NaCl added to diet	Chloride excretion (as NaCl)	Plasma chloride	Plasma urea nitrogen	Plasma creatinine	Urine formation	24-hour urea clearance	24-hour creatinine clearance
	grams per 24 hours	grams per 24 hours	m.eq. per liter	mgm. per cent	mgm. per cent	cc. per minute	per cent normal	cc. per minute
June 4....	10							
5....	10		106.1	50.9	6.95			
6....	10	7.9				1.16	4.7	
7....	10	8.3	106.1	42.4	6.3	1.18	5.6	10.4
8....	10	10.0				1.30	7.2	11.1
9....	10	11.5	106.6	38.0	6.1	1.39	7.8	10.7
10....	10	12.5				1.48	6.7	6.4
11....	0	15.8	107.1	34.6	6.1	1.77	8.3	11.9
12....	0	11.7	101.8	36.8	6.6	1.42	7.7	7.3
13....	0	9.0				1.22	5.4	8.5
14....	0	7.0				1.08	5.7	5.0
15....	0	5.6				1.01	5.7	8.3
16....	0	7.9	96.4	40.1	7.3	1.53	3.0	7.5



low protein diet and high fluid intake, adding salt freely to food during and after preparation.

Five months later the patient was again referred to the hospital on account of anorexia, nausea and slight weakness; she had not vomited and was ambulatory. On admission to the Renal Ward, plasma urea nitrogen was 161 mgm. per 100 cc., plasma chloride 94 m.eq. per liter, plasma creatinine 7.2 mgm. per 100 cc. and serum phosphates 7.3 mgm. per 100 cc. The results of clinical tests of renal function were essentially similar to those obtained during the previous admission. The patient was then submitted to rigidly controlled study as described under "Methods." The diet provided 2100 calories per day with 0.5 gram of protein per kilo. Fluid intake was kept rigidly at 4500 cc. per day, of which 3090 cc. consisted of 0.45 NaCl solution, so that 13.9 grams of salt were added daily to a diet which was of itself salt-poor, offering less than 1.0 gram of NaCl per day.

Figure 1 shows the plasma urea nitrogen (circles), plasma chlorides (crosses) and 24-hour urea clearances (dots) over a period of 53 days during which fluid intake was kept constant while dietary protein and salt were varied independently. To detect possible diurnal variations the urea clearances were computed for two 12-hour periods daily by means of the usual equations for "standard" and "maximal" clearances. Each dot represents, therefore, the average of one day and one night urea clearance. In the lower part of Figure 1, to make comparison easier, the rates of urine formation and urea clearances are shown also by shaded areas which represent average values over periods of 3 to 6 days. The urea clear-

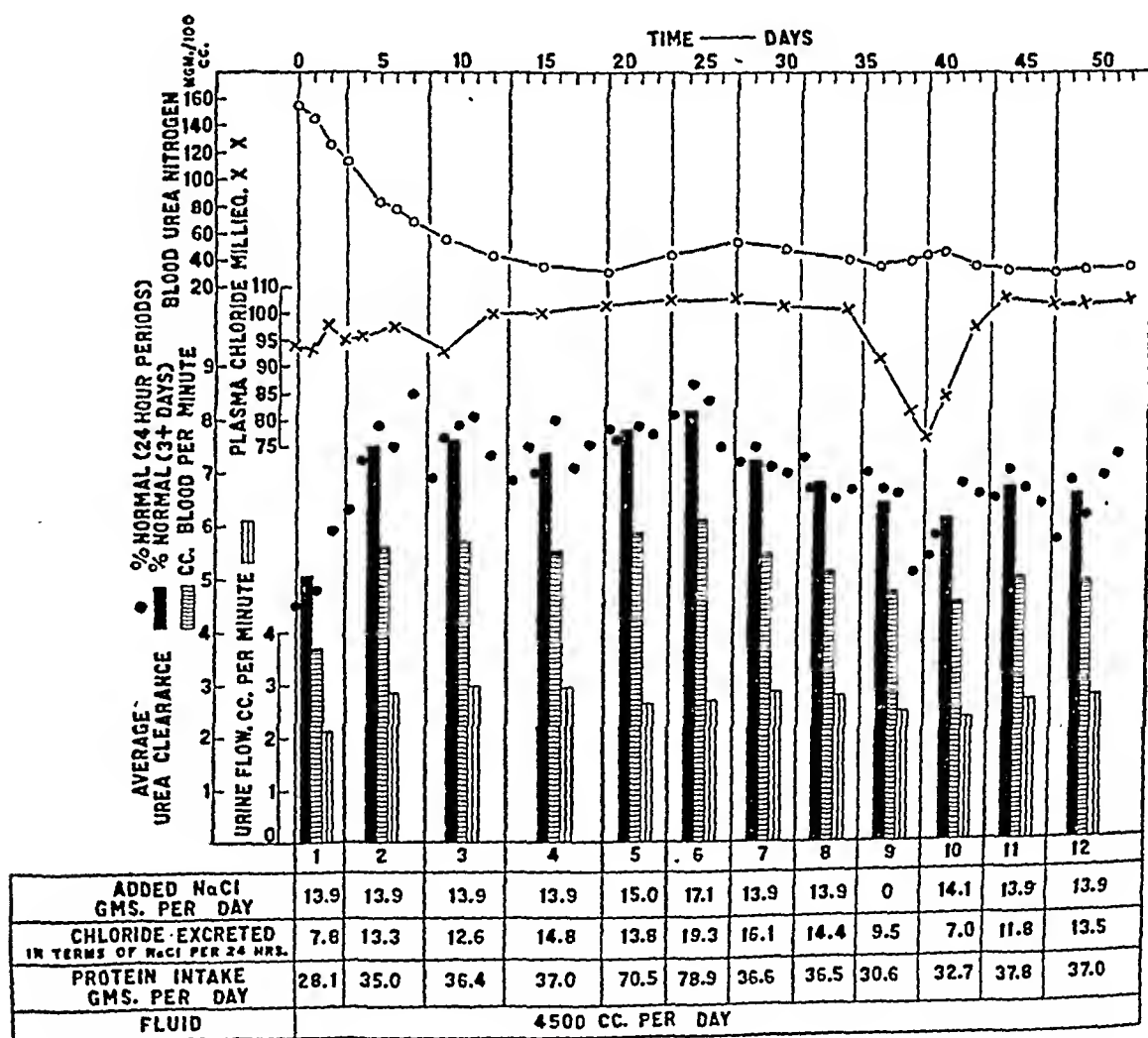


FIG. 1. CASE 1. SHOWING EFFECTS OF ADMINISTERING AND RESTRICTING SODIUM CHLORIDE IN A PATIENT WITH ADVANCED RENAL INSUFFICIENCY.

- Periods 1 to 4—low protein, high salt intake.
- Periods 5 and 6—increased protein, high salt intake.
- Periods 7 and 8—similar to a.
- Period 9—low protein intake with salt restriction.
- Periods 10 to 12—similar to a and c.

ances are expressed (a) in terms of cc. of blood cleared per minute (horizontal shading) and (b) in terms of per cent of normal (solid) using the Van Slyke equations.

The clinical course of this patient can be described best according to regime:

(a) *Low protein, high salt intake (Periods 1 to 4, duration 19 days)*

Although 37 grams of protein were offered in the daily diet, anorexia restricted the amount taken during the first 2 days so that in Period 1 the protein intake was only 28.1 grams per day. Thereafter the diet was taken almost completely as offered. Fluid intake was 4500 cc. per day from the start, but the urinary output during Period 1 (3 days) was only slightly above 2.0 cc. per minute. During this interval weight increased from 163 to 166 pounds and considerable chloride was retained as shown by the difference between the intake and excretion of chloride. After chloride balance was attained the rate of urine formation exceeded 2.5 cc. per minute and weight increased more slowly, reaching 167 pounds by the thirteenth day.

The plasma chlorides rose from 94 to 100 m.eq. per liter, plasma urea nitrogen fell from 154 to 32.8 mgm. per 100 cc., plasma creatinine from 7.2 to 5.0 mgm. per 100 cc., and serum phosphates from 7.3 to 4.9 mgm. per 100 cc. The 24-hour urea clearance rose abruptly during the first 5 days from 4.5 to 7 per cent of normal, averaging for the next 14 days 7.3 per cent of normal.

(b) *Increased protein, high salt intake (Periods 5 and 6, duration 8 days)*

The protein intake was increased from 0.5 to 1.0 gram per kilo and the salt intake was slightly increased while all other things were kept constant. The blood urea nitrogen, previously falling slowly but consistently, rose from 32.8 to 54.5 mgm. per 100 cc., plasma creatinine from 5.0 to 5.5 mgm. per 100 cc., and plasma phosphate from 5.0 to 6.9 mgm. per 100 cc. The urea clearances for these two 4-day periods (Numbers 5 and 6) were the highest observed in this patient.

Protein intake was thus increased in order to be certain (1) that the blood urea nitrogen was not falling merely because of high fluid intake

and (2) that the patient's inability to excrete nitrogen was demonstrable not only by a low urea clearance but also by a rising blood urea nitrogen on a liberal intake of salt with a diet which normally produces no azotemia.

(c) *Repetition of low protein, high salt intake (Periods 7 and 8, duration 8 days)*

Having ascertained that the patient could not excrete all the nitrogen arising from a protein intake of 1 gram per kilo daily, the dietary protein was reduced to the initial value of 0.5 gram per kilo, with 13.9 grams of salt per day.

The plasma urea nitrogen fell from 54.5 to 38.3 mgm. per 100 cc., and the 24-hour urea clearances again varied between 6 and 7 per cent of average normal.

(d) *Low protein, low salt intake (Period 9, duration 4 days)*

In this period no additional sodium chloride was administered. Activity, salt-poor diet and fluid intake were not changed. The plasma chlorides fell rapidly from 100 to 76 m.eq. per liter. Abundant chloride excretion continued, however, and weight decreased by 4 pounds in 3 days. The plasma urea nitrogen, which had previously been falling consistently, rose from 38.3 to 41.5 mgm. per 100 cc. Weakness, dizziness, anorexia and muscular stiffness developed on the third day. This was followed by nocturnal cramps in the calf muscles, generalized muscular stiffness on waking the next day, nausea, vomiting (only once) and some reluctance to take fluids and food. The anorexia is reflected in the lower protein intake during period 9.

On the last day of this period the total intake of fluid was 2500 cc.—the only instance in which fluid intake varied from 4500 cc. per 24 hours. Any changes in renal function cannot therefore be ascribed to dehydration in the ordinary sense of the word, nor to oliguria since the urinary output averaged more than 3000 cc. per 24 hours. Urea clearance, which had previously been dropping very slowly from the level attained during the period of increased protein intake, changed very little until the latter part of the period of restricted salt intake when the clearance dropped to a minimum figure of 4.3 per cent (see Table

TABLE IV

Case 1. Effect of sodium chloride restriction on urea and creatinine clearances with controlled salt-poor diet and high fluid intake

Date	NaCl added to diet	Chloride excretion (as NaCl)	Plasma chloride	Plasma urea nitrogen	Plasma creatinine	Rate of urine formation	12-hour urea clearance	12-hour creatinine clearance
	grams per 24 hours	grams per 24 hours	m.eq. per liter	mgm. per cent	mgm. per cent	cc. per minute	per cent normal	cc. per minute
December 4	13.9	14.0	101	40.0	5.5	2.38 3.04*	6.3 7.1*	10.4
5	0	12.1				2.74 2.63	7.3 6.7	10.6 7.8*
6	0	9.2	91	38.3	5.5	2.64 2.58	6.8 6.6	7.9 7.8
7	0	9.1				2.63 3.06	6.4 7.2	8.2 10.6
8	0	3.4	81	39.4	5.8	1.76 1.83	5.9 4.3	7.3 6.2
9	14.6	3.9	76	41.2	4.4	1.62 2.30	4.9 5.9	8.4 10.5
10	13.9	5.7	84	41.5	4.3	1.83 2.60	5.1 6.5	9.6 12.0
11	13.9	8.5				2.20 2.88	6.2 7.4	10.5 11.7
12	13.9	9.8	96	37.6	4.5	2.80 2.44	6.7 6.4	10.9 9.9
13	13.9	10.1				2.69 2.55	6.4 6.6	10.9 10.4
14	13.9	10.8	105	33.0	4.2	2.66 2.44	6.8 7.3	11.1 10.2
15	13.9	12.1				2.92 2.54	7.1 6.4	10.0 9.7
16	13.9	14.0				3.06 2.81	6.6 6.2	10.2 9.8
17	13.9	11.6	101	31.8	4.5	2.64 2.44	5.6 5.8	9.6 7.9
18	13.9	15.3				3.15 2.80	7.1 6.6	9.9 9.1
19	13.9	13.0	103	32.8	4.0	2.96 2.47	6.2 6.1	10.1 9.3

\* Lower figure of each pair applies to period from 8 p.m. to 8 a.m., upper to period from 8 a.m. to 8 p.m.

IV) for 12 hours, and an average of 5.1 per cent for 24 hours. This figure was lower than any observed during the 30 days immediately preceding Period 9 when sodium chloride was supplied freely.

It may be objected that the change in urea clearance appeared too late to be related to the restriction of sodium chloride. The patient lost 4 pounds of weight during Period 9, chloride excretion remaining above 9 grams per day until the

last day when it reached 3.4 grams per day. Contraction of body fluids would free sodium chloride for excretion, the effects of lack of sodium chloride appearing only when this compensation was exhausted. This lag of several days in the excretory manifestations of sodium chloride withdrawal and administration has already been noted (Rowntree and Fitz (1913), Vallery Radot (1918), Leiter (1926)). In Case 1 the urea clearance reached its earlier level of 6 to 7 per cent

only after 40 grams of salt had been given, this lag tending to make the average changes in urea clearances over 4 to 5 days (shaded areas, Figure 1) considerably less informative than the changes in consecutive 24-hour urea clearances.

*(c) Resumed low protein, high salt intake (Periods 10 to 12, duration 13 days)*

Without other change of regime sodium chloride was again administered in the dosage of 13.9 grams per day. The symptoms of hypochloremia disappeared within 3 days. Appetite returned, fluids were taken easily and muscular stiffness disappeared. Chloride was retained and body weight increased from 162 to 166 pounds in 4 days. The plasma chlorides returned to normal, the blood urea nitrogen ceased rising and fell gradually to 32 mgm. per 100 cc. The urea clearance, both in 24-hour periods and in the averages of 4 days (Period 10), was low while plasma chloride was still below normal. During the last 9 days (Periods 11 and 12) the urea clearance was once more between 6 and 7 per cent.

Chief interest attaches to the changes induced by restricting chloride intake in Period 9. Hypochloremia developed with surprising rapidity, and the symptoms produced were relieved equally rapidly as soon as sodium chloride was again administered. The symptoms resemble those produced by loss of sodium chloride (Moss (1923), Talbott and Michelsen (1933), Derrick (1934)). Blood pressure varied between 140/80 and 180/110, but no systematic relationship to salt administration could be detected. The patient was ambulatory throughout, except for the first 2 days of Period 1 and for several days in Periods 9 and 10, when weakness, dizziness and mental depression led to inactivity. On both occasions symptomatic improvement followed the administration of sodium chloride.

In this patient restriction of sodium chloride produced some of the effects stated to be characteristic of Addison's disease (Harrop et al. (1933)). Blood pressure was, however, never below normal and, during the period shown in Figure 1, was uniformly conspicuously above normal. There was no distinctive pigmentation of the skin or mucous membranes. Retrograde pyelography revealed normal, sharply defined

pelves and calices. Conspicuous renal insufficiency, organic in type, was the outstanding feature and persisted even when large doses of sodium chloride were given. If Addison's disease was present in addition it was not yet recognizable clinically.

Table IV presents a detailed comparison of 12-hour creatinine and urea clearances during Periods 9, 10 and 11. During the fourth day of salt restriction both urea and creatinine clearances reached their lowest levels. Creatinine clearances averaged 8.3 cc. per minute during the 4 days of salt restriction, and 10.1 cc. per minute when salt was administered. Creatinine clearance fell more rapidly and recovered more quickly after the restriction, and the administration respectively, of sodium chloride. The effect cannot be ascribed to changes in urine flow which have little or no influence on creatinine clearance (Holten and Rehberg (1931)). With respect to urea also there should be no grave error ascribable to changes in urine flow, since the rate of urine formation was relatively constant and since urea clearances were computed from the Van Slyke equations. Using the equation for "standard" clearance when the rate of urine formation was below 2.0 cc. makes the recorded differences in urea clearance less than they would have been if no correction had been made for diuresis. Hence any error in calculated urea clearances is in the direction of minimizing the changes under observation. Moreover, the observation described in Table IV agrees with that in Table III, though the rate of urine formation differed considerably in the two series. In this patient at two levels of diuresis, therefore, sodium chloride administration appeared to modify the facility with which urea and creatinine were excreted by markedly insufficient kidneys.

On discharge, the patient had no symptoms referable to azotemia; the plasma urea nitrogen was 34.6 mgm. per 100 cc., creatinine 4.7 mgm. per 100 cc., plasma chloride 100 m.eq. per liter, serum phosphates 5.9 mgm. per 100 cc. One month later, with 15 grams of sodium chloride per day, low protein diet and 3000 cc. of fluid per day, the plasma chlorides were 100 m.eq. per liter, the plasma urea nitrogen 27.8 mgm. per 100 cc., and the plasma creatinine 4.9 mgm. per 100 cc.

Case 2, F. M., white male, aged 45, was first admitted to the Medical Ward May 12, 1934. He presented gross

hematuria, edema, hypertension and azotemia. Phenolsulphonphthalein elimination was reduced at first to a trace in 2 hours. Diuresis set in and renal function improved steadily except for a brief period after tonsillectomy; on discharge a 2-hour urea clearance indicated 30 per cent of average normal function and phenolsulphonphthalein elimination was 25 per cent in 2 hours. He was discharged on July 26, 1934, with a diagnosis of subsiding acute glomerulonephritis.

was readmitted for further study. In the course of this second admission the relation between sodium chloride intake and urea clearance was studied.

The patient was ambulatory. A rigid salt-poor diet containing 2700 calories and 1.0 gram of protein per kilo daily was used. Fluid intake was kept at 3000 cc. per day throughout. Figure 2

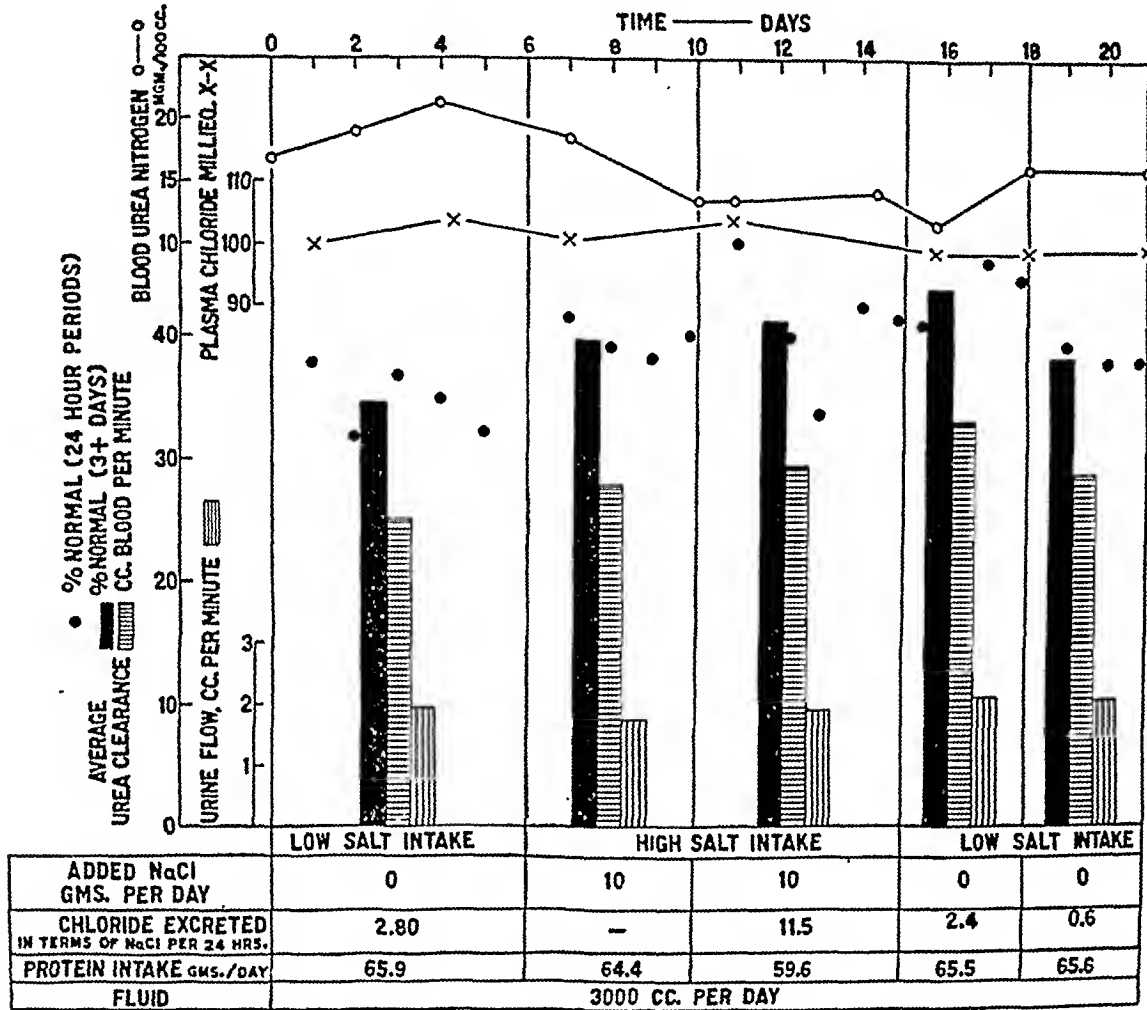


FIG. 2. CASE 2. SHOWING EFFECTS OF ADMINISTERING AND RESTRICTING SODIUM CHLORIDE IN A PATIENT WITH SLIGHT RENAL INSUFFICIENCY.

In October, 1934, a concentration test and cast count yielded the following results: Restriction of fluids for 24 hours (Addis) elevated the specific gravity of the urine to 1.018. The 12-hour volume was 720 cc., the reaction acid. Cast excretion totalled 79,000 per 12 hours with 45 per cent hyaline, 12 per cent granular, 10 per cent blood and 33 per cent epithelial casts. Erythrocyte excretion was 450 million per 12 hours, the urinary sediment being definitely red. Epithelial cells and leukocytes numbered 0.7 million in 12 hours. The plasma urea nitrogen was 16.6 mgm. per 100 cc. A 2-hour urea clearance showed 62 per cent of normal function, the plasma proteins were normal and the 12-hour specimen of urine contained 0.42 gram of protein. In view of persisting (active) subacute glomerulonephritis the patient

shows plasma urea nitrogen (circles), plasma chloride (crosses) and 24-hour urea clearances (dots) over 14 days. The shaded areas represent average urea clearance in terms of per cent normal function (solid), urea clearance in terms of cc. plasma cleared per minute (horizontal lines) and the average rate of urine formation (vertical lines).

For the first 6 days no salt was given except for that in the diet (less than 1.0 gram per day). Plasma chloride did not change, body weight diminished by 0.5 pound, and the average chloride

elimination was reduced to 2.80 grams per day. Average urea clearance was 34.7 per cent of normal and blood urea nitrogen rose from 16.9 to a maximum of 21.7 mgm. per 100 cc.

The administration of 10 grams of salt per day in the form of half-strength physiological salt solution was accompanied by a gain of 3.7 pounds in weight and by a fall of plasma urea nitrogen to 11.8 mgm. per 100 cc., with a definite increase of average urea clearance whether considered in terms of per cent normal, or simply in terms of cc. plasma cleared per minute. Protein intake was constant except for a period of slight anorexia during the last few days of high salt intake.

Reducing the sodium chloride intake, other things being constant, again diminished body weight by 3 pounds, the change being complete by the end of the third day of chloride restriction. The rate of urine formation increased during this time and the higher urea clearance observed may be due in part to the diuresis accompanying loss of body chloride and water. During chloride restriction the urine contained at first 2.4 grams of chloride (as NaCl) per day, and later 0.6 gram. In the latter part of the second period of chloride restriction the average urea clearance approached the level observed during the initial period of low salt intake. Plasma chlorides remained constant but the plasma urea nitrogen rose from 11.6 to 16.2 mgm. per 100 cc.

*Case 3, L. F., white male, aged 61, had been admitted to the hospital on several occasions since 1928 on account of marked hypertension (230 mm. Hg systolic and 140 mm. Hg diastolic), generalized arteriosclerosis, moderate obstructive symptoms from prostatic median bar (relieved in 1932 by cystoscopic resection) and chronic cystitis. A moderate grade of renal insufficiency was present. Phenolsulphonphthalein elimination amounted to 30 per cent in 2 hours.*

Restriction of fluids for 24 hours elevated the specific gravity of the urine to 1.017, the 12-hour volume being 855 cc. The blood urea nitrogen varied between 20 and 30 mgm. per 100 cc.; a 2-hour urea clearance revealed 35 per cent normal function. Creatinine clearance amounted to 44 cc. per minute. Plasma proteins were 8.07 per cent, and the urinary excretion of protein amounted to 0.13 gram per 12 hours. The excretion of many leukocytes, owing to cystitis, prevented computing erythrocyte and cast excretion.

This patient was studied for 14 days with the results shown in Figure 3, by symbols similar to

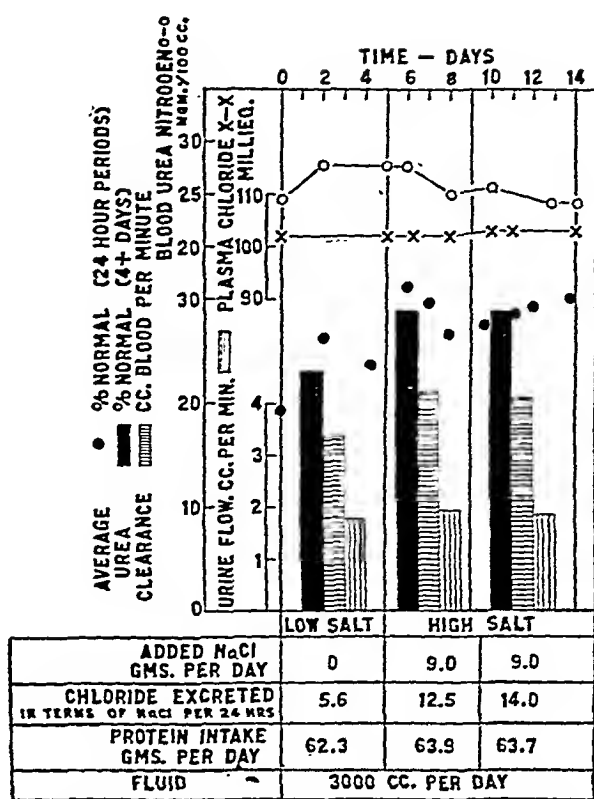


FIG. 3. CASE 3. SHOWING EFFECTS OF ADMINISTERING AND RESTRICTING SODIUM CHLORIDE IN A PATIENT WITH MODERATE RENAL INSUFFICIENCY.

those in Figures 1 and 2. The patient was placed on a diet containing 2500 calories and 1.0 gram of protein per kilo. Protein intake was practically constant, and fluid intake was kept to 3000 cc. per day. The diet was not so rigidly salt-poor, milk and salt-containing bread being allowed. The patient was ambulatory throughout.

During the first 5 days no salt was added to the diet, the output in the urine being 5.6 grams per day. Body weight was reduced by 1 pound. Blood urea nitrogen rose from 21.7 to 27.7 mgm. per 100 cc., remaining constant at the latter level. Plasma chloride was not altered. The 24-hour urea clearances averaged 23.5 per cent of normal.

During the succeeding 9 days, 9.0 grams of salt were added to the diet by substituting 2000 cc. of half-strength physiological salt solution for an equal amount of water in the fluid intake. Body weight increased by 2 pounds. The plasma urea nitrogen fell from 27.7 to 24.3 mgm. per 100 cc., the average urea clearance rising from 23.5 to between 29.2 and 28.9 per cent of normal during

TABLE V  
Summary of observations during NaCl administration and restriction

Case number	Period		Fluid	Protein	NaCl added to diet	Plasma urea nitrogen (end of each period)	Urine flow	24-hour urea clearance	Urea nitrogen excretion	Percentile change referred to Period 1 (= 100) in each case					
	Number	Duration								Salt output per 24 hours	Protein in diet	Urea nitrogen ex- cretion per 24 hours	Plasma urea nitro- gen (end of each period)	Cc. blood cleared of urea per min- ute	24-hour urea clear- ance (per cent normal)
		days	cc. per day	grams per day	grams per day	mgm. per cent	cc. per minute	per cent normal	grams per 24 hours						
1	0					154									
	1	3	4000	28.1	13.9	119	2.08	5.05	7.20	100	100	100	100	100	100
	2	5	4500	35.0	13.9	65.5	2.84	7.47	6.74	172	125	94	55	152	148
	3	5	4500	36.4	13.9	41.9	2.97	7.57	4.20	163	130	58	35	154	150
	4	6	4500	37.0	13.9	33.5	2.93	7.33	2.94	191	132	41	28	149	145
	5	4	4500	70.5	15.0	41.9	2.63	7.78	3.14	178	251	44	35	159	154
	6	4	4500	78.9	17.1	53.3	2.69	8.15	4.22	249	281	59	45	166	161
	7	4	4500	36.6	13.9	45.6	2.84	7.19	3.90	207	130	54	38	147	142
	8	4	4500	36.5	13.9	38.3	2.79	6.81	3.10	186	130	43	32	138	135
	9	4	4065	30.6	0	41.5	2.48	6.41	2.68	122	109	37	35	130	127
	10	4	4500	32.7	14.1	36.5	2.33	6.14	2.58	90	116	36	31	123	122
	11	4	4500	37.8	13.9	32.0	2.71	6.68	2.38	152	135	33	27	136	132
	12	5	4500	37.0	13.9	34.6	2.74	6.52	2.34	174	132	33	29	133	129
2	0					16.9									
	1	5	3000	65.9	0	19.3	1.96	34.7	7.24	100	100	100	100	100	100
	2	4	3000	64.4	10	14.2	1.78	39.8	6.64		98	92	74	111	115
	3	5	3000	59.6	10	13.0	1.94	41.2	5.82	411	90	80	67	117	119
	4	3	3000	65.5	0	15.1	2.13	44.0	6.20	87	99	86	78	131	127
	5	3	3000	65.6	0	15.7	2.07	38.3	6.64	21	99.5	92	81	114	110
3	0					24.6									
	1	5	2975	62.3	0	27.7	1.75	23.5	6.42	100	100	100	100	100	100
	2	4	3000	63.9	9.0	25.2	1.94	29.2	7.82	223	103	122	91	126	124
	3	5	3000	63.7	9.0	24.3	1.84	28.9	7.24	247	102	113	88	122	123

salt administration. Mild grades of salt restriction and administration were used since the marked hypertension made it inadvisable to test extreme conditions. Severe headache complained of before admission disappeared during salt restriction, but returned in mild grade during the last few days of salt administration.

The important average figures for each of the 3 cases are summarized in Table V. Absolute values are given in the left hand portion of the table. Percentile changes during the period of study are shown at the right; for quantitative comparison the various values applying to the first period for each case have been taken as 100 per cent, each subsequent period being compared to Period 1.

In Case 1 salt excretion was doubled at the time urea clearance was highest (66 per cent above the urea clearance for Period 1). The times in which urea clearance was lowest (Periods 1, 9 and 10) were those in which salt excretion was least. Urea nitrogen excretion was greatest shortly after admission (7.20 grams per 24 hours) falling to a minimum of 2.34 grams per 24 hours in Period 12, showing no definite relation to urea clearance. It would seem that the excessive excretion of urea nitrogen during the early periods resulted simply from the elimination of retained urea. The elimination of urea nitrogen was finally reduced to 33 per cent of the initial value, and the blood urea nitrogen was reduced to 29 per cent of the initial value.



Protein intake was approximately constant in Case 1, except for Periods 1 and 9, owing to anorexia, and Periods 5 and 6 when protein intake was purposely increased. The observed reduction in urea clearance and elevation of blood urea nitrogen cannot be explained by the slight change in protein intake during the low salt period. The similarity in the percentile changes in urea clearance (per cent of normal) and cc. of plasma cleared of urea per minute indicates that changes in the rate of urea excretion are demonstrable by either method of calculation and do not depend upon the more or less arbitrary use of the square root of the rate of urine formation contained in Van Slyke's equation for calculating standard clearances. This equation was used in isolated instances in Periods 1, 2, 5, 9 and 10. In other periods the clearances were maximal since the average rate of urine formation was usually above 2.0 cc. per minute.

In Case 2 chloride excretion was increased fourfold in Period 3 and urea clearance was 17 per cent higher than in Period 1 when salt intake was low. The total urea excretion was lower, however, partly by reason of voluntarily reduced protein intake. As in Case 1, moreover, the effects of salt restriction were not evident immediately. Loss of weight showed that body fluid was being diminished. The sodium chloride thus made available might delay for some days the effects of chloride restriction. Similar lag in the excretory response to the administration and restriction of chloride is described by Rowntree and Fitz (1913), Vallery-Radot (1918) and by Leiter (1926) who emphasize the importance of body fluids and tissue in connection with salt balances. Our own observations with chloride-poor diets in patients with edema indicate that the urinary excretion of chloride remains high until the edema fluid has been excreted. Only after weeks will reduction in chloride intake produce in edematous patients the reduction of urea clearance that is observed in non-edematous individuals after 4 to 5 days.

In Case 3 protein intake was more constant and the increased urea clearance was associated also with increased total excretion of urea nitrogen, while salt output was doubled.

The studies of 3 patients with different grades

of renal insufficiency agree in showing during salt administration (1) a tendency toward lower plasma urea nitrogen, (2) a slight increase in the volume of plasma cleared of urea per minute and (3) slightly increased urea clearance over 12- and 24-hour periods when calculated by the Van Slyke equation in terms of per cent of normal.

#### *Urea clearances and diuresis during day and night*

In the 3 patients with renal insufficiency urine was collected in two 12-hour periods extending from 8 p.m. to 8 a.m., and from 8 a.m. to 8 p.m., in order to detect any large differences in urea clearance that might be related to diurnal change in the rate of urine formation, or to diurnal changes in excretory activity. The 12-hour urea and creatinine clearances shown in Table IV do not show any striking or uniform relation to the period of urine collection. It should be mentioned, however, that while urea clearances varied irregularly, creatinine clearances were lower by night in 11 of 15 periods, and higher by night in the remainder, though the differences were extremely slight.

The data on Cases 1 and 2 are charted in Figure 4, to compare the rate of urine formation and urea clearance in night and day periods. Solid dots refer to the night period from 8 p.m. to 8 a.m., circles to the day period from 8 a.m. to 8 p.m. The charts show no systematic relation between day and night urea clearances, the distribution of points being in general haphazard.

The rate of urine formation in Case 1 tended to be very slightly higher during the night, but the variation was slight as is usually the case in renal insufficiency. There was no uniform relation between urea clearance and the diuresis. This is to be expected since clearances were, with two exceptions, maximal (i. e., associated with urine formation at rates exceeding 2.0 cc. per minute). While salt was being given, the rate of urine formation was occasionally less than 2.0 cc. per minute (Figure 4, Periods 2 and 5) but the urea clearance in terms of per cent of normal remained at the usual level. In Case 1 the low clearances associated with salt restriction were accompanied by slightly lower rates of urine formation, the lowest corresponding to over 2300 cc. per 24 hours. Since occasional relative oliguria in



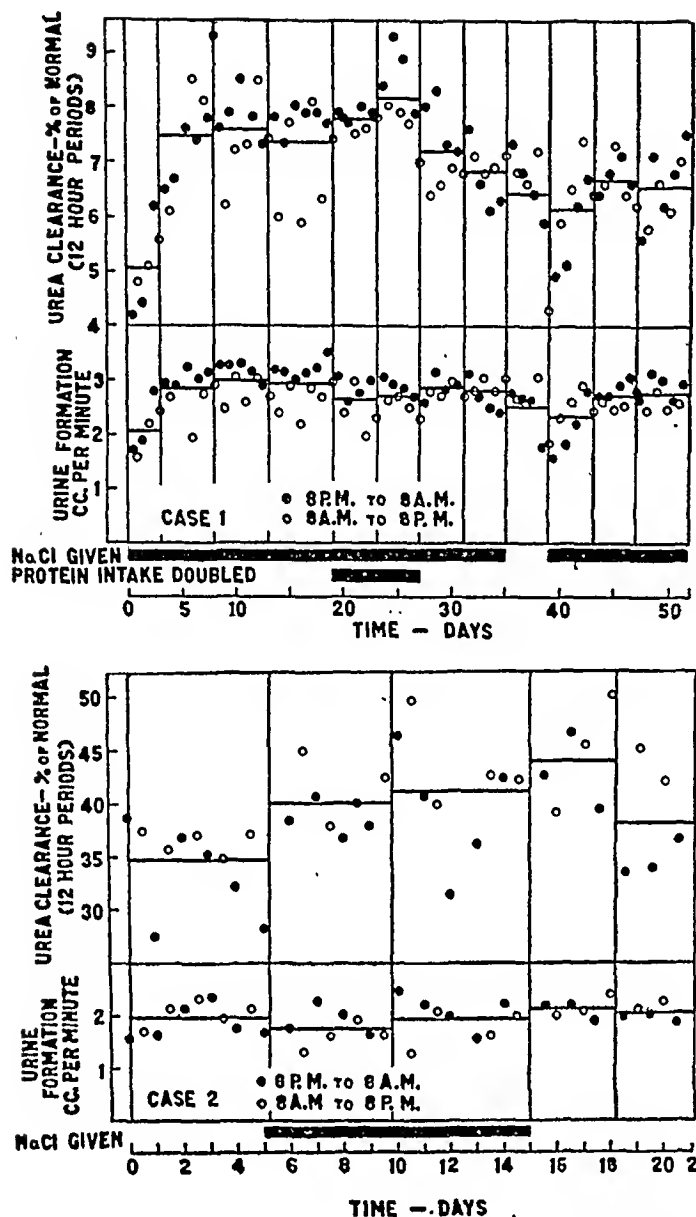


FIG. 4. CASES 1 AND 2. SHOWING UREA CLEARANCE (PER CENT NORMAL) AND DIURESIS DURING DAY (8 A.M. TO 8 P.M., CIRCLES) AND NIGHT (8 P.M. TO 8 A.M., DOTS) PERIODS.

the periods of salt administration did not affect urea clearance measurably, it seems most unlikely that the low urea clearances observed during hypochloremia were secondary to relative oliguria alone.

In Case 2 (Figure 4) diuresis was relatively constant, while 12-hour clearances varied more widely. There were no definite diurnal variations. The diurnal fluctuations in renal excretion described by Simpson (1926), Norn (1929) and Manchester (1932) while conspicuous, were of short duration and would in large measure be neutralized by 12-hour periods of collection, particularly since the night specimens included the

urine formed immediately before and after sleep. Manchester (1932) found nitrogen excretion very slightly greater in the daytime, but stated that the increase in specific gravity of the night urine was due largely to nitrogenous substances in spite of the fact that the total amount of urinary nitrogen excreted was slightly smaller during the night. Moreover, it is to be expected that patients with renal insufficiency would not show significant diurnal variations in excretion since the disappearance of nocturnal relative oliguria is one of the first indications of renal insufficiency.

#### DISCUSSION

A number of clinical reports indicate that chloride administration may be helpful in reducing azotemia in selected cases (Vallery-Radot (1914), Romalo and Dumitresco (1914), Blum et al. (1929), Borst (1931)), though adverse effects from the administration of large doses of sodium chloride have also been observed (Veil (1918), Lemierre et al. (1929)). The findings in Case 1 resemble those of a case described by Chabanier et al. (1934). A patient with chronic glomerulonephritis showed vomiting, convulsions and oliguria, associated with a blood urea of 542 mgm. per 100 cc. and a blood chloride of 250 mgm. per 100 cc. The administration of sodium chloride diminished the oliguria and eventually lowered the blood urea to 25 mgm. per 100 cc. Renal function in Chabanier's patient was 10 per cent of normal, according to the Ambard coefficient.

In agreement with the data of McLester (1922) and of Allen and Sherrill (1922) it appears that restriction of sodium chloride may be associated with elevation of plasma urea nitrogen with or without lowering of plasma chlorides. The observations made during the administration of salt agree in general with those of Hatcher and Sollmann (1902) and Schoenthal (1929). If the changes in 24-hour urea clearances described for the three patients studied prove to be significant, it would seem that the greater variability of 2-hour urea clearances will explain the negative results of Leiter (1926) and Cope (1933). The wide range of normal variation often observed in short period studies of renal function may have concealed the relatively slight changes in urea clearance required to produce the cumulative

effects that are represented by a slight elevation of the concentration of blood urea nitrogen. It may be added that 2-hour urea clearances, determined in the course of our studies of 3 patients, did not show changes which could be ascribed to differences in salt intake; during the same period the 24-hour urea clearance indicated the small, but apparently consistent, changes recorded.

Chaussin (1920) found that when fluid is restricted the administration of chlorides in large doses diminishes the excretion of urea. Davies, Haldane and Peskett (1922) observed that the maximal possible molecular concentration of chloride in the urine is 0.33 normal, and that the total molecular concentration of chlorides plus bicarbonate cannot exceed this figure. They believed, however, that the excretion of urea and chlorides were not interdependent. According to Adolph (1923) the total molecular concentration of the urine does not ordinarily exceed a fixed value, and if the concentration of one substance approaches this value in the urine the excretion of other substances will be depressed. These findings are not opposed to our own, since fluid intake was liberal enough to avoid the excretion of highly concentrated urine. The amount of fluid was graded so that the specific gravity of the urine was below the maximum characteristic for each patient. Under these conditions the antagonism described above could not be an important limiting factor.

Our observations yield no specific information concerning the importance of the total osmotic concentration of the blood in determining nitrogen retention. The finding of a measurable, though small, change in urea clearance suggests, however, that depressed renal function per se may play a part in the development of azotemia in those conditions which are characterized by the loss of large amounts of chloride.

The identification of the exact mechanism by which renal function can be reduced during chloride restriction must await further opportunity to study other patients with hypochloremia and conspicuous azotemia. In Case 1 the reduced intake of sodium chloride apparently tended to diminish both the urea and creatinine clearances. It is known that loss of chloride produces dehydration and anhydremia; plasma volume is reduced and

the concentration of plasma protein is increased (Veil (1918)). It is conceivable that the anhydremia of salt restriction may affect creatinine clearance in the same way that the anhydremia of standing does. According to Ni and Rehberg (1931) creatinine clearance and glomerular filtration are less in the standing position because blood volume is reduced and plasma protein concentration increased, by filtration of relatively protein-free filtrate from the blood stream into the dependent parts of the body. The coincident depression of urea and creatinine clearances suggests that reduced glomerular filtration may be in part responsible. A definite decision on this point cannot be made with the limited data now available. The results thus far obtained are described since this method of study may help in determining the mechanism through which depletion of sodium chloride favors the development of azotemia in certain patients.

#### SUMMARY

In 2 normal subjects urea clearances determined over 24-hour periods varied less widely than did the conventional urea clearances determined over 1-hour periods. Average urea clearances over 12- and 24-hour periods were therefore used to study possible effects of sodium chloride restriction and administration on renal function in 3 patients with different grades of renal insufficiency.

When diet and fluid intake were kept constant, a restricted intake of sodium chloride was accompanied by a slight elevation of plasma urea nitrogen concentration and by a slightly diminished average 24-hour urea clearance. Administration of sodium chloride was accompanied by a lowering of plasma urea nitrogen concentration and by a higher average 24-hour urea clearance.

In one patient with advanced renal insufficiency the plasma urea nitrogen decreased from 154 to 26 mgm. per 100 cc. during sodium chloride administration. Acute restriction of sodium chloride intake produced hypochloremia with characteristic symptoms. In addition, there was temporary retention of urea, creatinine and phosphates. Renewed sodium chloride administration relieved the symptoms and diminished the concentrations of urea, creatinine and phosphate in the plasma.

In this same patient both the urea and the creatinine clearances over 24-hour periods were slightly lower during and shortly after sodium chloride restriction, becoming somewhat higher during sodium chloride administration.

It is suggested that this method of study applied to suitable cases may aid in determining the factors responsible for the association, in certain patients, of hypochloremia and azotemia.

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# VITAL CAPACITY OF THE LUNGS: CHANGES OCCURRING IN HEALTH AND DISEASE

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In 1854 Andrews (1), of the University of Michigan, wrote of the spirometer "... I am inclined to adopt the opinion of others who maintain that this instrument cannot be relied upon for diagnosis except where the previous vital capacity of the individual is known, so as to decide whether there is really a diminution going on. At the same time it must be confessed that diseases of the lungs exert a remarkable influence on the capacity." One year before this, Fabius (2), in his inaugural dissertation on the vital capacity of the lungs, had also suggested making determinations during health in order that, should sickness supervene, the former might serve as a basis of comparison with that secured after the onset of disease. However, these suggestions have largely gone unheeded, most of the publications from the time of Hutchinson (3) to the present being concerned with the variations from standards based upon body measurements rather than with actual changes in the vital capacity, incident to disease.

In 1924, with the publication with Kornblum (4) of an article on the vital capacity, the writer decided to employ the suggestions of Fabius and Andrews, and see whether the value of the vital capacity as a diagnostic procedure would be enhanced thereby. It was decided that an attempt should be made to secure the vital capacity of every patient presenting himself for office treatment, regardless of the nature of his complaint, and that, in addition, the vital capacity of certain available groups of healthy individuals should be studied.

## *Vital capacity changes in youthful healthy subjects*

The first group to be considered consisted of 482 healthy students in Drexel Institute, Philadelphia. (See Table I.) Half of them were men ranging in age from 17 to 30 years with an av-

erage age of 21.36 years, the other half were women, aged 16 to 25, the average age being 20.14 years.

TABLE I  
*Ages of normal subjects*

Age years	Men	Women
16 .....	0	1
17 .....	1	4
18 .....	23	24
19 .....	47	68
20 .....	53	82
21 .....	43	37
22 .....	42	18
23 .....	19	5
24 .....	7	1
25 .....	3	1
26 .....	2	0
27 .....	0	0
28 .....	0	0
29 .....	0	0
30 .....	1	0
Total .....	241	241
Mean age ....	21.36 years	20.14 years

In this group the vital capacity figures of one year were compared with those of the next. It will be seen (Fig. 1) that many variations occurred both above and below the readings of the previous year; that both men and women exhibited an average gain, and that the men gained more than the women. The gain of the men exceeds three times the standard error of the mean and is statistically significant, that of the women is not.

It was interesting to compare these findings with the variations from the hypothetical vital capacity as calculated from the standards of West (5) which are based upon surface area. As would be expected, the variations from West's standard (Fig. 2) were considerably greater than those from the reading of the previous year, showing that the latter offered a better basis for predicting the vital capacity than did West's surface area standard.

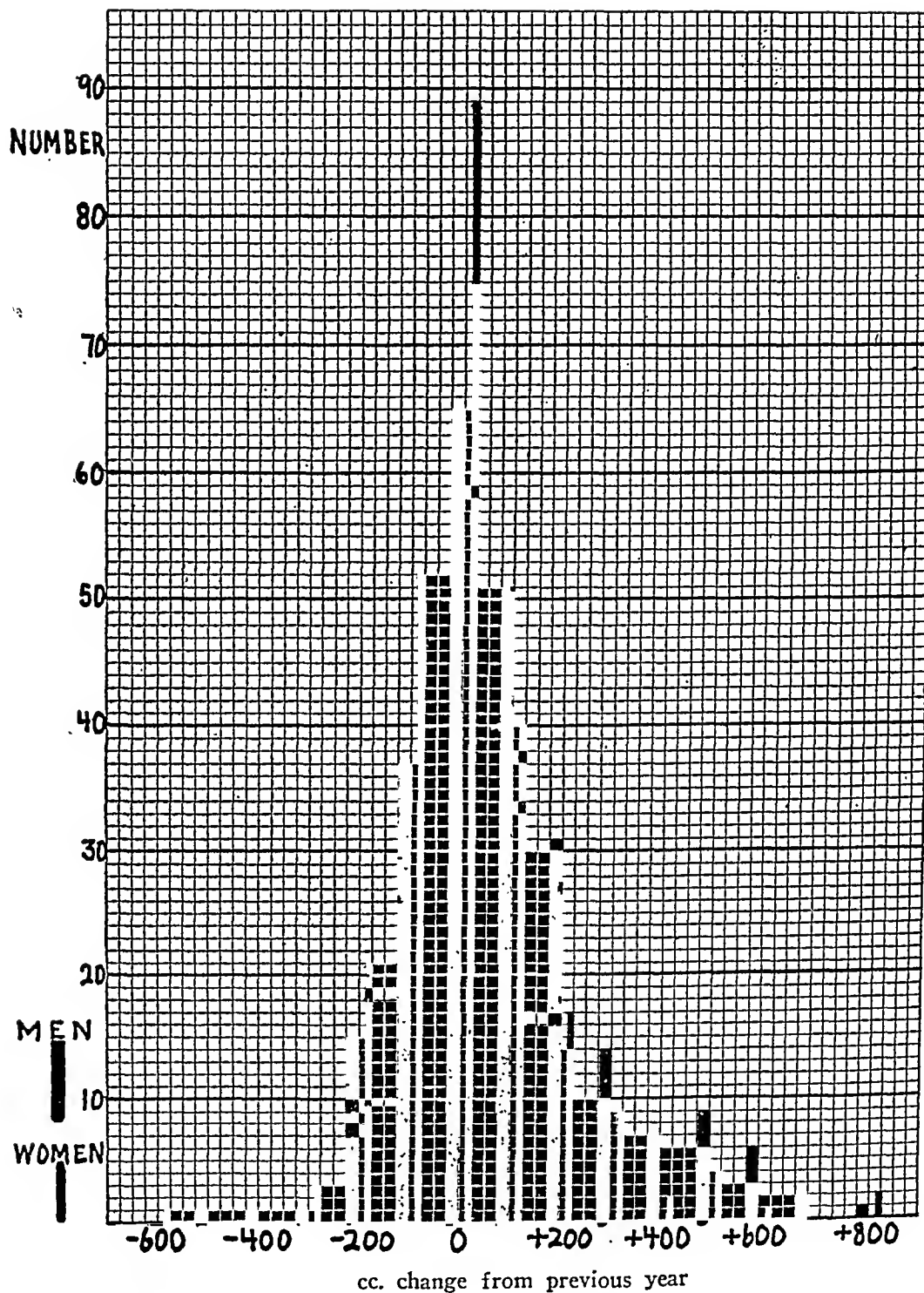


FIG. 1. VITAL CAPACITY CHANGES FROM PREVIOUS YEAR IN 482 NORMAL SUBJECTS.

Standard deviation = 202 cc. for men, 169 cc. for women. Standard error of the mean = 13 cc. for men, 11 cc. for women. Mean gain in vital capacity = 89 cc. for men, 17.4 cc. for women.

The data, furthermore, help to answer the question: "How far below the normal figure can the vital capacity fall before the diminution should be regarded as significant?" Adopting three times the standard deviation as the criterion of significance in the case of the student whose vital capacity is compared with that of the previous

year, a diminution exceeding 695 cc. for the men and 507 cc. for the women would be regarded as significant.<sup>1</sup> On the other hand, where a student's

<sup>1</sup> For the men, the standard deviation (202 cc.)  $\times$  3 = 606 cc. Adding the mean gain (89 cc.) gives 695 cc. For the women, the standard deviation (169 cc.)  $\times$  3

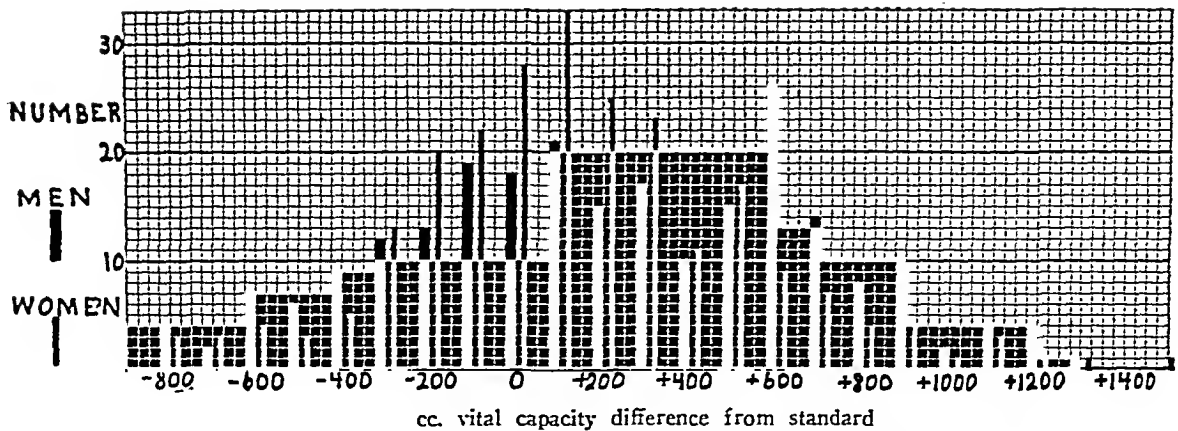


FIG. 2. VITAL CAPACITY VARIATIONS FROM WEST'S STANDARD IN 482 NORMAL SUBJECTS.

Standard deviation = 482 cc. for the men, 385 cc. for the women. Standard error of the mean = 31 cc. for the men, 25 cc. for the women. Mean difference from West's standard = +198 cc. for the men, +137 cc. for the women.

vital capacity is compared with the figure calculated by West's formula, readings more than 1,644 cc. below West's standard in the case of men, and 1,292 cc. below in the case of women may be considered significant.<sup>2</sup>

#### *Vital capacity changes in acute bronchitis*

Having determined what variations were to be expected in the vital capacity of youthful subjects in health, a group of youthful patients with acute bronchitis was next studied. A difference of opinion regarding the effects of bronchitis upon the vital capacity is manifested in the literature, some writers believing that little if any diminution is produced, while others hold that a marked lowering of the vital capacity is to be expected. Table II shows these patients divided into ambulatory and bed cases. As might be expected the bed cases exhibited a greater average loss than the ambulatory ones. From a statistical standpoint the loss in vital capacity in the ambulatory group could not be regarded as significant; in the bed cases, however, the difference is almost 3 times

TABLE II

#### *Vital capacity changes in acute bronchitis* (Cases grouped as ambulatory or bed)

Vital capacity difference from healthy figure cc.	Number of ambulatory patients	Number of bed patients
+400 .....	1	0
+300 .....	1	0
+200 .....	4	2
+100 .....	11	4
0 .....	13	3
-100 .....	9	6
-200 .....	9	5
-300 .....	0	2
-400 .....	1	1
-500 .....	1	0
-600 .....	0	1
Total .....	50	24
Standard error of the mean .....	23.2 cc.	38 cc.
Standard error of the mean $\times 3$ .....	69.6 cc.	114 cc.
Mean loss in vital capacity .....	20 cc.	100 cc.

the standard error of the mean, and probably is significant. However this may be, a study of Table II indicates that in most instances the vital capacity would have afforded little assistance in differentiating bronchitis from normality. On the other hand, this very fact indicates that the vital capacity might be of assistance in differentiating that disease from others which regularly cause a significant diminution in the vital capacity, pneumonia, for example.

#### *The vital capacity changes in pneumonia*

Although it has long been known that the vital capacity is greatly diminished in pneumonia, yet,

gives 507 cc. (the gain is not significant and is therefore not added).

<sup>2</sup> For the men, the standard deviation (482 cc.)  $\times 3$  gives 1,446 cc. Adding the mean difference from West's standard (+198 cc.) gives 1,644 cc. For the women, the standard deviation (385 cc.)  $\times 3$  gives 1,155 cc. Adding the mean difference from West's standard (+137 cc.) gives 1,292 cc.



except in postoperative pneumonia (6) vital capacity determinations prior to and during the onset of this disease have heretofore been lacking. In Cases 1 and 2 it was possible to secure fairly complete sets of vital capacity readings, before, during, and after pneumonia. (See Figs. 3 and 4.)

the physical signs were by no means characteristic of pneumonia, there being skodaic resonance over the right base, little if any alteration in the breath sounds and few râles over the right lower lobe. The patient never looked acutely ill, and she improved daily so that on the 14th day of the disease a roentgenogram showed the chest to be entirely negative, and she was discharged. Twelve days later she returned to her normal activities.

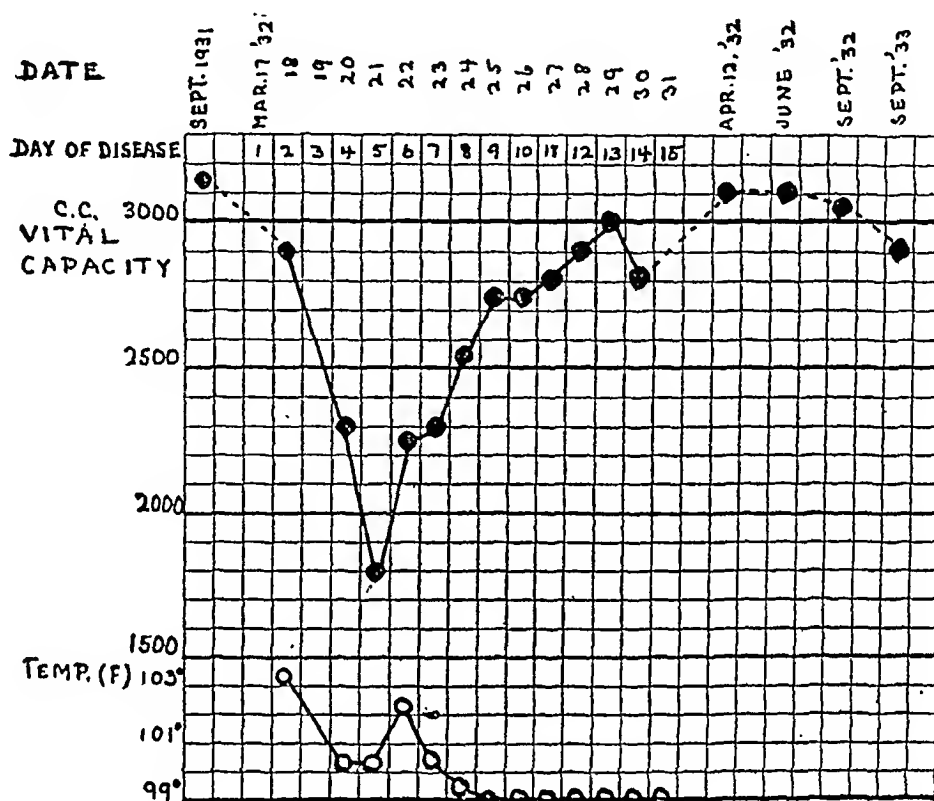


FIG. 3. VITAL CAPACITY BEFORE, DURING AND AFTER MILD INFLUENZAL PNEUMONIA (CASE 1, FIG. 5A).

A loss of 250 cc. by the second day of the disease is shown, then a further loss of 1,100 cc. followed by a rapid rise approximately to the figure secured prior to the illness. Here, and in Figure 4, the highest temperature of each 24 hours is indicated by a circle.

*Case 1.* Miss V. B., aged 20, suffered from unreduced congenital bilateral dislocations of both hips: otherwise her past medical history was negative. Her vital capacity in health was found to be 3,100 cc. (See Fig. 3.) Six months later she was seized with pain in the abdomen and back, and on the following day she developed a chill, cough and fever of 102.8° F. The chest examination was negative. On the next day her leukocyte count was 8,500 and râles appeared at the base of the right lung. Because of the fall in her vital capacity, and despite the atypical findings, pneumonia was diagnosed and she was sent to the University Hospital. On the 6th day of her disease her leukocytes numbered 19,600 and a roentgenogram showed "density at the right base, the appearance of which is that of pneumonic consolidation." (See Figs. 3 and 5A.) Even at this time

*Case 2.* Miss M. S., aged 20, in health had a vital capacity of 3,500 cc. Six months later she was seized with a sore throat and a fever of 103° F. On the following day she had a leukocytosis of 11,400 and examination of the chest revealed the classical signs of pneumonia over the lower lobe of the right lung. She was taken to the University Hospital where she ran a course characteristic of lobar pneumonia, the upper as well as the lower lobe becoming involved by the pneumonic process (see Figs. 4 and 5B). On the 33d day of the disease she was discharged from the hospital, and 29 days later resumed her normal activities.

These cases illustrate the diminution in vital capacity which regularly accompanies pneumonia. In Case 1 the fall was gradual and the disease

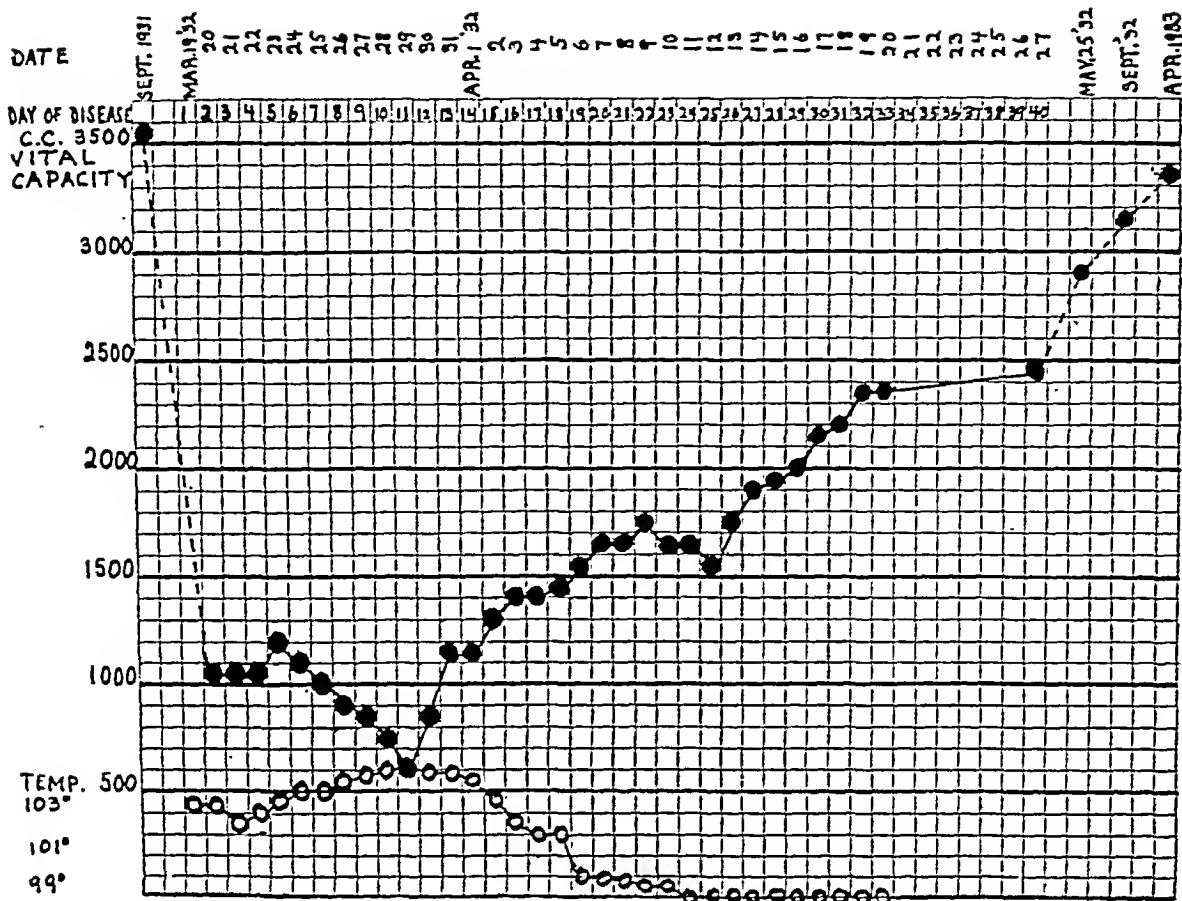


FIG. 4. VITAL CAPACITY BEFORE, DURING AND AFTER LOBAR PNEUMONIA INVOLVING FIRST THE RIGHT LOWER, THEN THE RIGHT UPPER LOBE OF CASE 2 (FIG. 5B).

A loss of 2,500 cc. by the second day of the disease is shown, then a further loss of 450 cc., followed by an increase beginning before subsidence of the temperature has begun. The vital capacity 1 year and 7 months after the attack is shown to be very little below that secured prior to its onset.

mild. Here the vital capacity was of distinct service, strongly suggesting the diagnosis of pneumonia. It is possible that cases of mild pneumonia masquerading as bronchitis may be more frequent than is generally recognized, and if so, the vital capacity should assist in their recognition. Case 2 exhibited a more precipitous and profound diminution in the vital capacity than did Case 1. The physical findings and course of the disease were so characteristic of lobar pneumonia that neither the vital capacity nor roentgenogram were needed to make the diagnosis. It is interesting to note how early in the disease the fall in the vital capacity occurred and how an increasing vital capacity was the first evidence of beginning lysis. Both in this patient and in Case 1 there

was eventually a return of the vital capacity to approximately the pre-pneumonia level. That such does not always occur is evidenced by the following case.

*Case 3.* A man, aged 40, measuring 70½ inches and weighing 145 pounds, whose vital capacity in health was 6,000, developed a prolonged attack of streptococcal bronchopneumonia which resulted in the diaphragmatic adhesion shown in Figure 5C. Although in perfect health during the subsequent 5 years the vital capacity has remained 5,500 cc.

In spite of the loss of 500 cc. his vital capacity is still 1,000 cc. above the normal figure as predicted on the basis of West's standard. This case illustrates how a complication such as pleuritic adhesions may lead to a permanent diminution in the

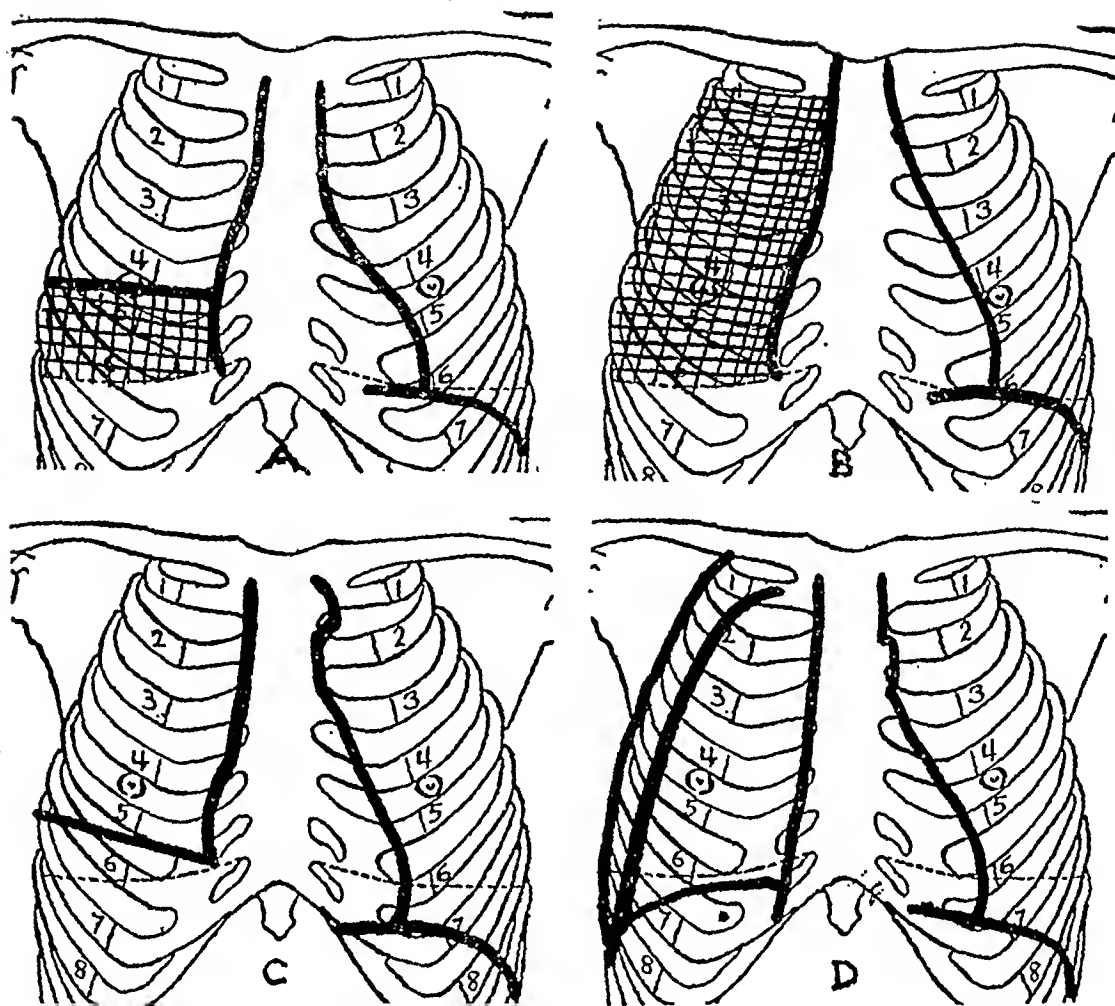


FIG. 5. ROENTGENOGRAPHIC FINDINGS.

A. Case 1. Mild atypical pneumonia involving the right lower lobe. The vital capacity loss was 1,350 cc. (see Fig. 3).

B. Case 2. Right upper and lower lobe pneumonia. The vital capacity loss was 2,950 cc. (see Fig. 4).

C. Case 3. Adherent right diaphragm due to preceding bronchopneumonia resulting in a permanent loss of 500 cc. in vital capacity.

D. Case 4. Spontaneous pneumothorax of right lung. The vital capacity loss was 500 cc.

vital capacity and how relying upon West's standard one might conclude that the vital capacity was above normal whereas a loss of 500 cc. is known to have occurred.

#### *Vital capacity changes in spontaneous pneumothorax*

The literature contains a number of observations upon patients under treatment with artificial pneumothorax in whom vital capacity determinations were made both before and after the introduction of air (7). The following case, however, is apparently the first in which the vital capacity before and after the occurrence of a spontaneous pneumothorax is reported.

Case 4. Mr. C. S., aged 20, consulted the writer on March 21st, 1928, because of pain over the right eye which appeared following swimming. The positive physical findings were: blood pressure 140/85, left tonsil red, right frontal sinus dark on transillumination. His vital capacity at this time was 4,300 cc. Two weeks later he presented himself perspiring, pale and complaining of pain in the right chest which was aggravated by breathing or coughing. The onset was sudden and followed moderately violent exertion. The breath sounds over the right chest were slightly diminished but other chest findings, including the coin test, were negative. The vital capacity was found to be 3,800 cc., a loss of 500 cc. from that of 2 weeks before. The roentgenogram (see Fig. 5D) revealed partial collapse of the right lung due to pneumothorax. One month later the vital capacity had returned to within 100 cc. of the original figure, the

chest examination was negative, and the roentgenogram showed complete expansion of the affected lung.

### *Vital capacity in heart disease*

Although it has been recognized since the time of Kentisch (8) that congestive heart failure caused marked diminution of the vital capacity, yet, as far as the writer is aware, Case 5 is the first in which the vital capacity before, during and after the first attack of decompensation is reported.

*Case 5.* Mr. W. H., aged 57, consulted the writer in August, 1928, because of pharyngitis. His vital capacity was 3,400 cc. (900 cc. below the figure predicted from West's surface area standard.) He gave a history of having had a retinal hemorrhage 6 years before and of hypertension for an indefinite period, but he had never suffered from cardiac decompensation. He was not heard from again until December 22d of the same year, at which time he suffered from cough and dyspnea on exertion, his vital capacity being 2,900 cc. His heart, which previously had shown little or no enlargement, now extended well outside the mamillary line and a systolic murmur had become audible over the apex. An electrocardiogram made at this time revealed inverted T waves in Lead I, diphasic T waves in Lead II and notched QRS waves in Lead III. The cough grew worse and on January 11th, 1929, he was orthopneic, the vital capacity being 1,800 cc. This represented a loss of 1,100 cc. in 20 days and a total loss of 1,600 cc. since August. Under treatment in the hospital he gradually improved and on February 11, 1931, cardiac compensation was well established and his vital capacity was 3,500 cc.

This case is of interest in showing the gradual diminution of the vital capacity incident to decompensation and the return of the vital capacity to its original level as compensation becomes restored.

### SUMMARY

1. The vital capacity in two consecutive years was determined in a group of 482 youthful

healthy subjects. Deviations from the readings of the previous year proved to be decidedly smaller than those from the hypothetical vital capacity as calculated from the surface area formula of West. The reading of the previous year, therefore, constituted the more reliable basis for vital capacity prediction.

2. In a group of 74 cases of acute bronchitis an average diminution from the previously determined healthy figure was noted in both ambulatory and bed cases, a more marked diminution occurring in the latter.

3. The vital capacity is shown before, during and after illness, in cases of pneumonia, spontaneous pneumothorax and cardiac decompensation. These observations are believed to be unique.

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# STUDIES ON THE MECHANISM OF THE INCREASED OXYGEN CONSUMPTION IN PATIENTS WITH CARDIAC DISEASE

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Since the introduction of accurate methods for measuring oxygen consumption, that function has been repeatedly observed to be increased in persons with congestive heart failure (1, 2, 3, 4) and to decline as improvement occurs (4). The mechanisms underlying these changes have not been clearly defined. In studying a group of patients with heart failure we were impressed with the constancy and magnitude of the alterations in metabolic rate and attempted to identify some of the factors responsible for them.

This group of patients consisted of individuals with heart disease due to hypertension, syphilis or rheumatic infection. Most of the patients were moderately decompensated, a few had severe failure and a smaller number were without congestive phenomena or had symptoms due to neuroses. The usual measures used in treating heart failure were employed, i.e., rest, sedation, digitalis, diuretics and limitation of fluids. Venesection, thoracentesis and abdominal tapping were resorted to as occasion demanded. Observations were made at frequent intervals under the usual "basal" conditions with the patient sitting in a wheel chair. The oxygen consumption was measured by gas analysis of the expired air, duplicate determinations being made as a rule. The metabolic rates were calculated on the basis of the edema-free weight, using the Boothby and Sandiford modification of DuBois' standard tables (16). In order to minimize the factor of nervousness and apprehension the subjects were trained for one or two days prior to the beginning of the observations.

Twenty-five patients were studied. They have been classified in three groups, depending on the response to treatment. Group I consists of 13 individuals who showed marked improvement as evidenced by three criteria, i.e., a loss of weight of at least 5 pounds, an increase in vital capacity of 10 per cent or more and the disappearance of paroxysmal dyspnea. Most of the subjects in

this group had moderate or advanced congestive failure at the beginning of the observations. In Group II are six persons who claimed subjective relief but in whom the objective evidences of improvement, although demonstrable, were not sufficient to meet the standards for Group I. In general, the patients in this group were not as severely decompensated as those in Group I. Those in Group III showed either doubtful or no improvement and include the patients with well compensated heart disease or with symptoms due to neuroses.

The results are shown in Table I. The data recorded are the initial level of the metabolic rate and the change in the rate as improvement occurred. In all but two patients the metabolic rate was 10 per cent or more above the calculated normal, when congestive failure was present, and tended to decline with improvement. The highest metabolic rates were found in Group I, where they ranged between 15 and 60 per cent above normal, with an average of 32 per cent above normal. The greatest change in rate with improvement in the clinical state was noted in this group, the average decrease being 22 per cent and the range between 5 and 39 per cent. The patients in Group II showed smaller but qualitatively similar changes. In Group III the basal metabolic rates were, with one exception, within the normal range and no striking variations were observed over periods of a week or longer in the hospital. These observations are in agreement with those reported by previous investigators.

Among the factors that may be considered as conceivably responsible for the increased oxygen consumption in cardiac failure are:

1. Fever
2. Psychic disturbances such as fear or tenseness
3. Altered activity of the thyroid gland
4. An enlarged capillary bed secondary to increased venous pressure
5. Increased work of the respiratory muscles

TABLE I

*The basal metabolic rate at the beginning of observation and the changes in metabolism and  $\frac{\text{Ventilation}}{\text{Vital capacity}}$  with improvement \**

	Name	Initial level of basal metabolic rate	Change in basal metabolic rate with improvement	Change in $\frac{\text{Ventilation}}{\text{Vital capacity}}$ with improvement
		<i>per cent</i>	<i>per cent</i>	
Group I Moderate or severe cardiac failure, marked improvement	M.T.	+60	-39	-4.2
	Y.D.	+37	-30	-1.6
	P.F.	+52	-30	-1.0
	M.L.	+34	-28	-2.0
	A.M.	+37	-28	-1.9
	A.M.†	+35	-18	-0.6
	J.E.	+15	-26	-0.9
	E.P.	+30	-22	-1.4
	J.K.	+32	-20	-1.2
	I.M.	+26	-20	-1.0
	W.C.	+24	-15	-1.0
	F.B.	+30	-15	-4.0
	A.E.	+15	-5	-1.1
	G.M.	+23	-5	-0.2
	Average	+32	-22	-1.6
Group II Slight improvement. Slight cardiac failure	C.S.	+55	-30	-2.0
	R.M.	+15	-20	-0.2
	M.B.	+18	-20	-0.5
	P.F.	+10	-10	-0.6
	A.C.	+5	0	-0.4
	L.M.	+16	0	-0.4
	Average	+20	-15	-0.7
Group III No improvement. No cardiac failure	J.M.	-3	+6	-0.3
	R.O.	-10	0	-1.0
	S.F.	+5	0	+0.5
	M.W.	-6	0	-0.6
	E.O.	+23	-4	+2.3
	U.R.	+2	0	+0.2
	Average	+5	0	+0.5

\* The degree of the decline in metabolism and in respiratory effort appear to vary directly with the extent of improvement.

† Second admission.

## 6. Increased oxygen consumption of the heart itself.

The first factor need not be considered in the present study since none of our subjects had fever at the time of observation. The five other factors suggested may be considered separately.

### *Psychic disturbances*

Emotional disturbances will vary in different individuals and are to some extent unavoidable. We tried to minimize them by using well trained persons, many of whom had been subjects for

respiratory observations on previous admissions. The most obviously neurotic patients were found in Group III, but in this group the metabolic rates remained fairly constant, so that we felt that variations due to psychic factors could not account for the striking changes observed in the other groups.

### *The factor of altered activity of the thyroid gland*

Lev and Hamburger (4) suggested that an altered activity of the thyroid gland secondary to circulatory changes may be responsible for the increased metabolism in patients with cardiac disease. The known circulatory changes in congestive failure are a prolongation of the circulation time, and an increase in venous pressure. An attempt was made to produce similar changes in the neck veins of dogs either by suspending the animal upside down or by applying a pressure cuff inflated to 40 mm. Hg. about the neck below the level of the thyroid gland. In several animals so treated over a period of 4 or 5 hours no significant variations in oxygen consumption were observed.

A favorable response to iodine has been considered a good index of the presence of thyrotoxicosis. One patient with severe congestive failure and a basal metabolic level of +35 did not respond to iodine administration over a period of two weeks—although on the usual cardiac regime he had repeatedly shown marked improvement and a decline in basal metabolic rate to a level of about +10 per cent. This evidence, though meager, does not favor the hypothesis of a transient hyperthyroidism in cardiac failure.

### *The effect of an enlarged capillary bed*

Could the increased oxygen consumption observed in congestive failure be due to the increased venous pressure *per se*? Such a change might conceivably follow the enlarging of the area of contact between tissues and blood as a result of dilatation of the venules and capillaries. Attempts were made to test this hypothesis by measuring the oxygen consumption in a limb or isolated muscle under varying conditions of venous pressure. In some experiments an elevation in metabolism was noted, accompanied by either an increase or decrease in blood flow. In others the opposite effect was found. There were many

technical difficulties and no procedure was free from theoretical objections, so that we cannot draw any conclusions from our data.

Observations of interest in this connection have been made in four patients with increased venous pressure, peripheral edema, ascites and hepatomegaly, the results of obstruction to the entrance of blood into the heart. The obstruction in one case was due to a mediastinal tumor, and in the other three to pericarditis (*concretio cordis*). Dyspnea at rest was not a prominent symptom and the hearts were either normal in size or only slightly enlarged. The levels of the basal metabolic rates in these cases were respectively +9 per cent, -9 per cent, -11 per cent and -15 per cent. In the last subject following resection

of a portion of the pericardium the venous pressure declined from a level of 400 to 200 mm. H<sub>2</sub>O without any accompanying change in oxygen consumption. This evidence suggests that increased venous pressure of itself cannot be responsible for elevation of the metabolic rate.

*The factor of increased work of respiratory muscles*

Peabody, Meyer and DuBois made the first satisfactory determinations of the basal metabolic rate in patients with heart disease, and noted an increase in oxygen consumption in dyspneic patients (1). They stated, "Dyspneic patients must do an increased amount of work in their labored breathing but it is doubtful if this would account

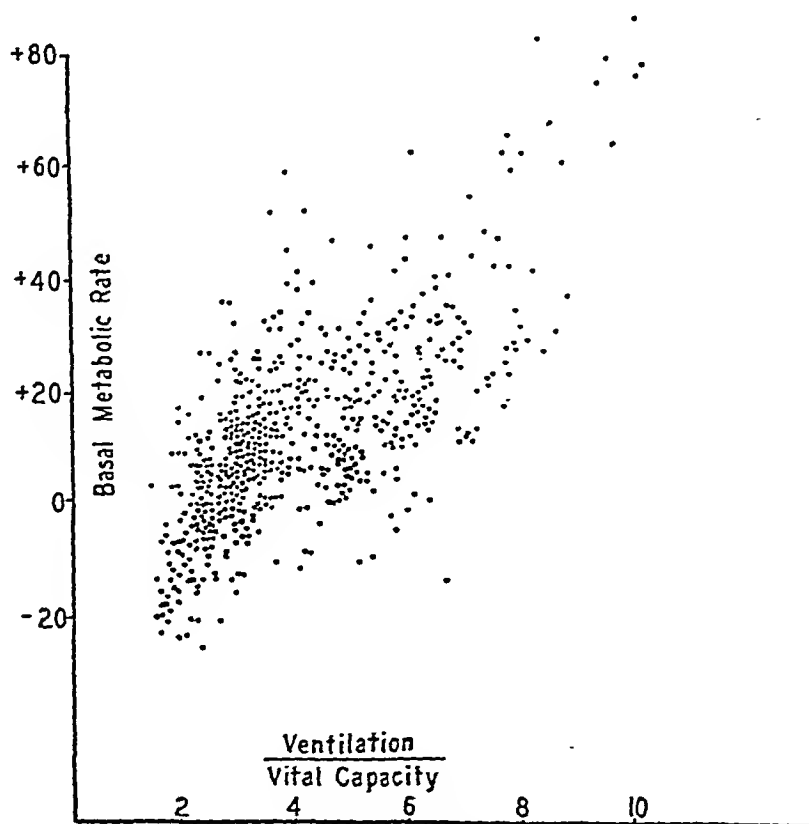


FIG. 1. RELATION OF B.M.R. TO  $\frac{\text{VENTILATION}}{\text{VITAL CAPACITY}}$  OF MISCELLANEOUS PATIENTS WITH HEART DISEASE IN VARIOUS STAGES OF COMPENSATION.

This chart represents repeated measurements on 25 patients with congestive failure. The basal metabolic rate is plotted against respiratory effort as measured by the ratio  $\frac{\text{Ventilation}}{\text{Vital capacity}}$ . Although there is considerable scattering of points there is a correlation between the functions studied.



for an increase of more than 10 per cent." Peabody, Wentworth and Barker stressed the importance of the reduced vital capacity and the increased ventilation in heart failure and recognized their relation to dyspnea and the increased oxygen consumption (2). These ideas were emphasized

with the volume of air breathed and inversely as the vital capacity.

In normal people under basal conditions the values for  $\frac{\text{Ventilation}}{\text{Vital capacity}}$  have been found to be usually less than 2. In patients with heart dis-

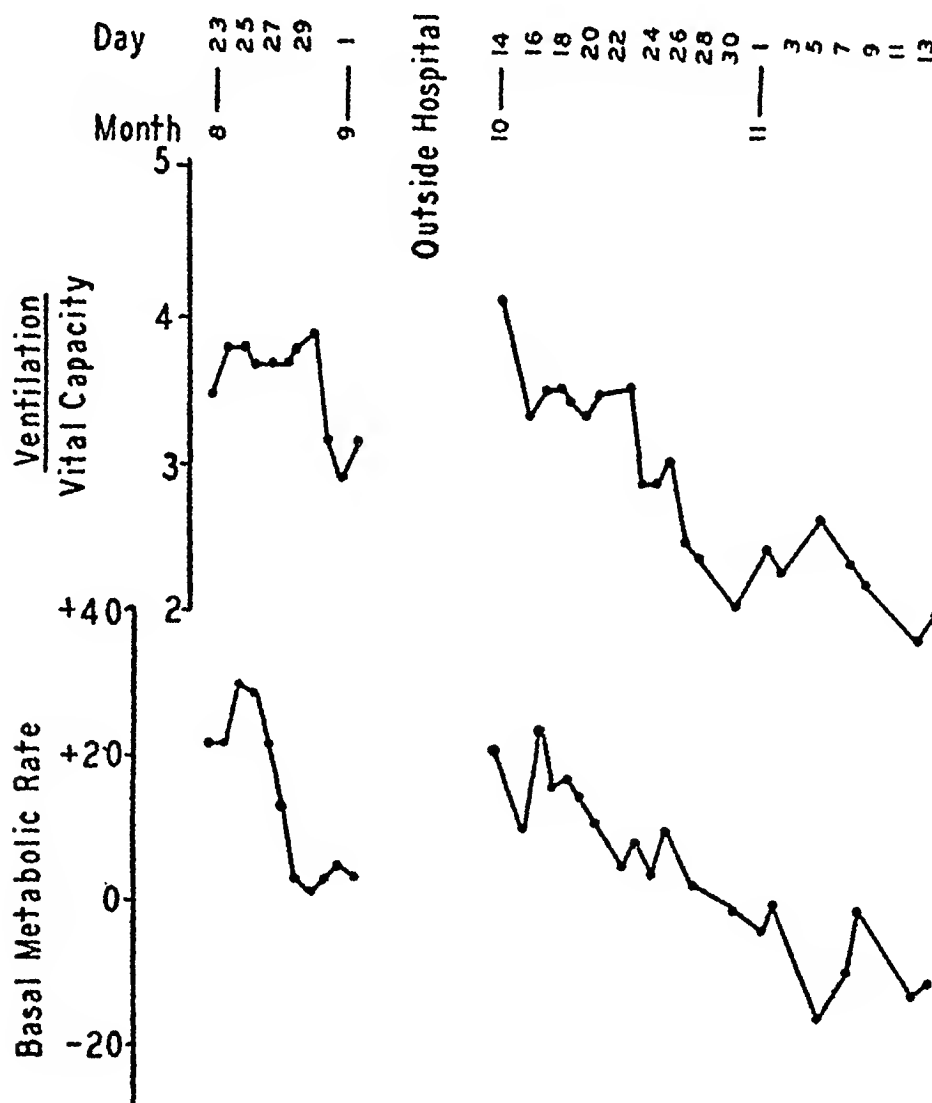


FIG. 2. CHANGES IN B.M.R. AND  $\frac{\text{VENTILATION}}{\text{VITAL CAPACITY}}$  IN PATIENT A.M. DURING TREATMENT FOR CONGESTIVE FAILURE.

This illustrates the rough parallelism between changes in the basal metabolic rate and the amount of dyspnea as represented by the ratio of  $\frac{\text{Ventilation}}{\text{Vital capacity}}$  during recovery from congestive failure on two occasions.

by Harrison and his coworkers (5), who found the degree of dyspnea to be roughly proportional to the expression  $\frac{\text{Ventilation (in liters per minute)}}{\text{Vital capacity (in liters)}}$ .

This ratio may be used to represent also the work performed by the muscles of respiration which, other things being equal, will tend to vary directly

ease values ranging up to 10 have been noted. In Figure 1 are represented more than 300 observations of the basal metabolic rate and the ratio  $\frac{\text{Ventilation}}{\text{Vital capacity}}$  in 25 patients in various stages of cardiac failure. The points show considerable scattering. There is, however, an apparent cor-

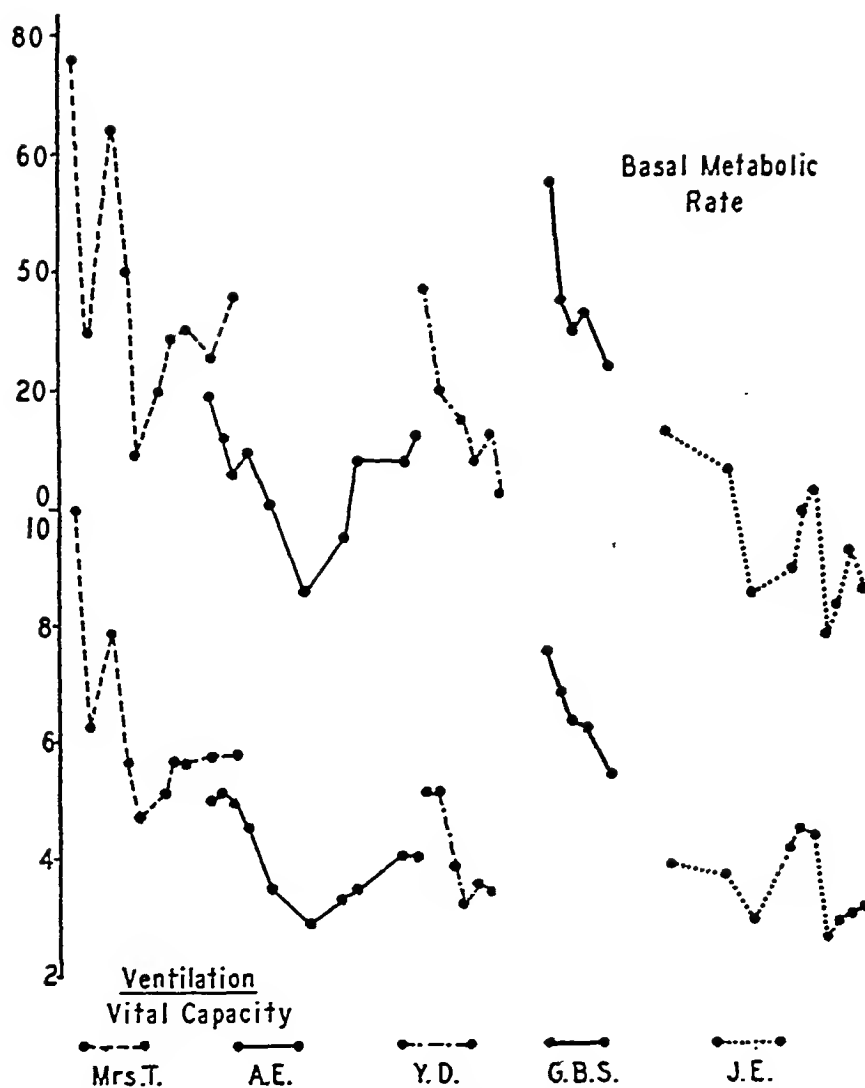


FIG. 3. CHANGES IN B.M.R. AND  $\frac{\text{VENTILATION}}{\text{VITAL CAPACITY}}$  IN CARDIAC CASES UNDER TREATMENT.

This chart illustrates the general parallelism between variations in the basal metabolic rate and the ratio of  $\frac{\text{Ventilation}}{\text{Vital capacity}}$  in five patients during recovery from congestive failure.

relation between the two functions studied, particularly when the ratio for the  $\frac{\text{Ventilation}}{\text{Vital capacity}}$  exceeds 6. The data on two patients with congestive failure associated in one with mild thyrotoxicosis, and in the other with postoperative myxedema, gave values for these individuals that fell along the upper and lower limits of the graph, respectively. The numerical changes in the ratio

$\frac{\text{Ventilation}}{\text{Vital capacity}}$  in the group of patients studied are recorded in the last column of Table I. There was a decline in this function in all persons who showed definite improvement. The diminution was greatest in Group I, in which as we have noted, failure was most severe, improvement well defined, and reduction in oxygen consumption most marked.

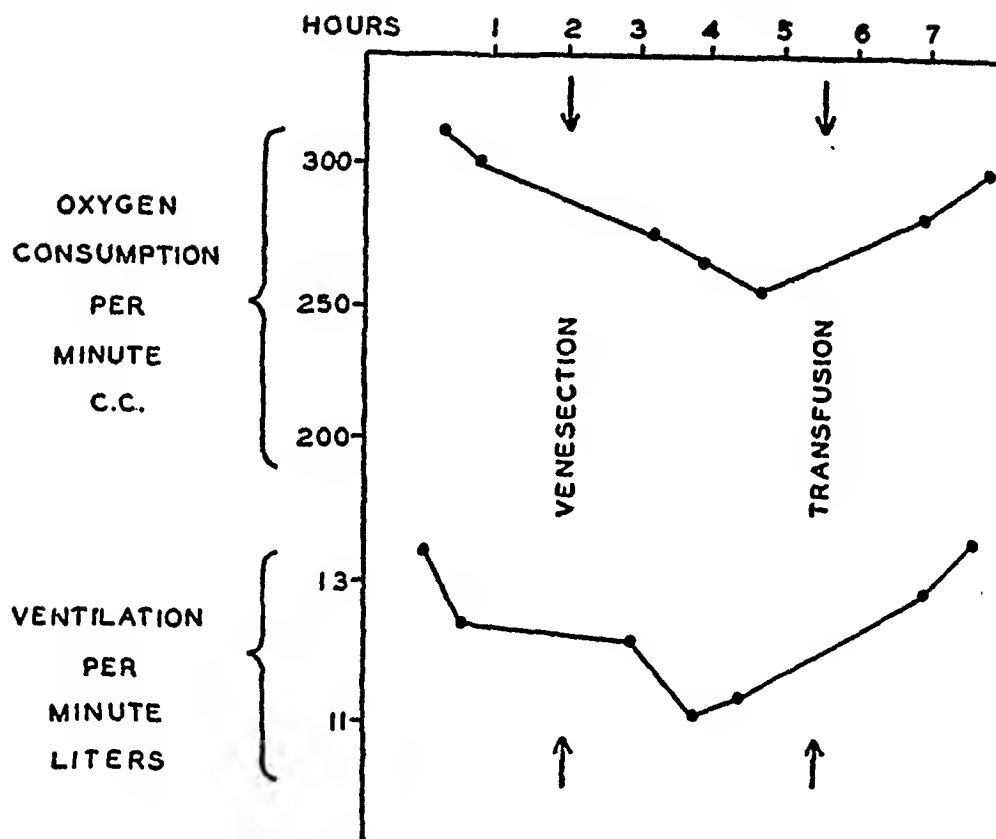


FIG. 4. SUBJECT F. B. EFFECT OF VENESECTION AND TRANSFUSION ON METABOLISM AND VENTILATION.

After venesection of 500 cc. of blood there was a fall in the oxygen consumption and in the ventilation of the patient. After the blood was reinfused the oxygen consumption and ventilation both rose.

The correlation noted above is more striking in the graph for the individual subject. In charting the serial changes as the patient improves the tendency to parallel variation is again brought out. In Figure 2 are represented the data on Patient A. M. during two periods of hospitalization, and in Figure 3 studies over shorter periods on other persons with heart disease. At times the metabolic rate and the ratio for the  $\frac{\text{Ventilation}}{\text{Vital capacity}}$  tend to vary independently, or one may lag behind the other, indicating that there are other factors to be considered.

Sudden and parallel changes in oxygen consumption and total ventilation were observed following abdominal paracentesis in a patient with rheumatic heart disease and massive ascites (Table II). About 4 liters of fluid were removed. Observations were made just before the operation, immediately afterward, 6 hours and 24 hours later. There was an immediate drop of 28 per cent in ventilation and 23 per cent in the oxygen consumption, which was fairly well sus-

TABLE II

*Effect of abdominal paracentesis on metabolism and ventilation.*

(Subject A. T.: Rheumatic heart disease, congestive failure with ascites. Four liters of fluid removed at one sitting.)

	Before paracentesis	After paracentesis	Per cent change
Vital capacity, cc.	1850	*	?
Ventilation, liters per minute.....	10.7	7.7 Immediately after 6.3 Six hours later 7.4 Next day	-28 -41 -31
Oxygen consumption, cc. per minute.....	202	157 Immediately after 161 Six hours later 169 Next day	-23 -20 -16

\* On account of pain at the site of incision the vital capacity after operation could not be determined.

tained for at least 24 hours. On account of pain at the site of incision the vital capacity after abdominal tapping could not be determined.

The effect of venesection is illustrated graph-

ically in Figure 4. The initial oxygen consumption was 310 cc. per minute. Following the removal of 500 cc. of blood there was a drop in the oxygen consumption to 260 cc. per minute in a period of two and one half hours. An hour later the blood was reinfused, after which the oxygen consumption rose almost to its original level. The changes in ventilation parallel closely the variations in metabolism. Comparable diminutions in metabolism were observed in 3 other patients in whom venesection was carried out. In only one instance was the blood reinfused.

panied by an increase in the rate of oxygen consumption. There have been identified a number of variables among which are the rate and depth of respirations, the level of ventilation and the presence or absence of respiratory obstruction. Loewy (6) reviewed the earlier investigations on this subject. Lev and Hamburger (4) noted a rise of 17.5 per cent in the metabolic rate in one patient during a period of voluntary hyperpnea. Anrep and Hammouda (7) found in panting dogs that the oxygen consumption increased in proportion to the total ventilation and concluded that the

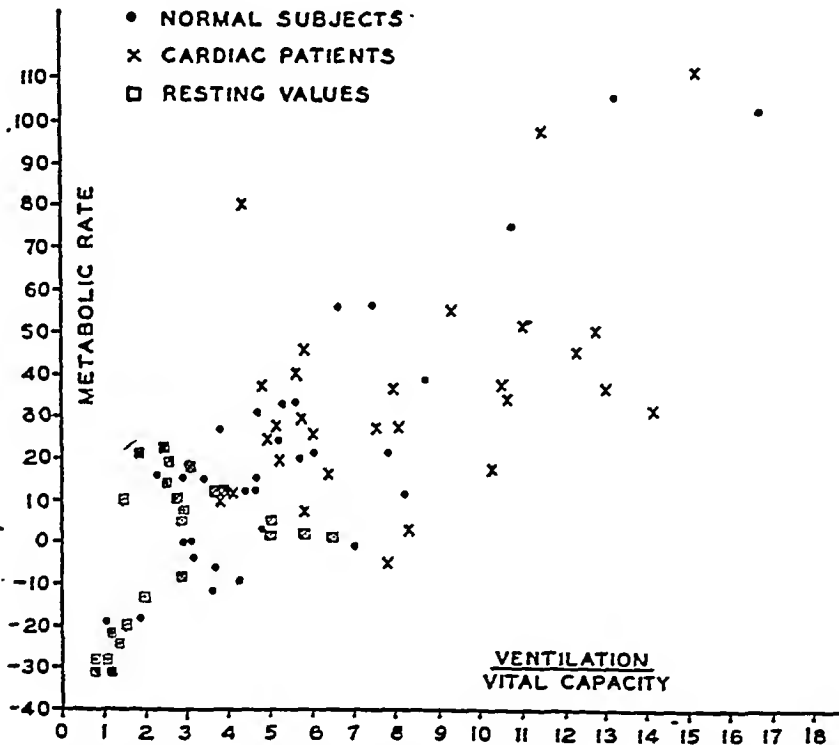


FIG. 5. THE EFFECT OF VOLUNTARY OVERVENTILATION ON THE METABOLIC RATE.

Seven normal individuals and eight patients with cardiac disease were studied. The points that are squared represent resting values. The others were obtained during voluntary overventilation. The chart illustrates the extent to which an increase of ventilation can raise the metabolic rate.

The data so far presented indicate that there is a qualitative, and to some extent quantitative, correlation between the metabolism and the work of the respiratory muscles (as measured by the expression  $\frac{\text{Ventilation}}{\text{Vital capacity}}$ ).

There are several reports in the literature indicating that an increase in the ventilation is accom-

panied by an increase in the rate of oxygen consumption.

Marked elevations in metabolism were observed in subjects made to overventilate either voluntarily or by breathing carbon dioxide. Figure 5 presents composite results in 7 normal people and 8 patients with cardiac disease during voluntary hyperpnea. No attempt was made to regulate the rate or depth of breathing. The total period

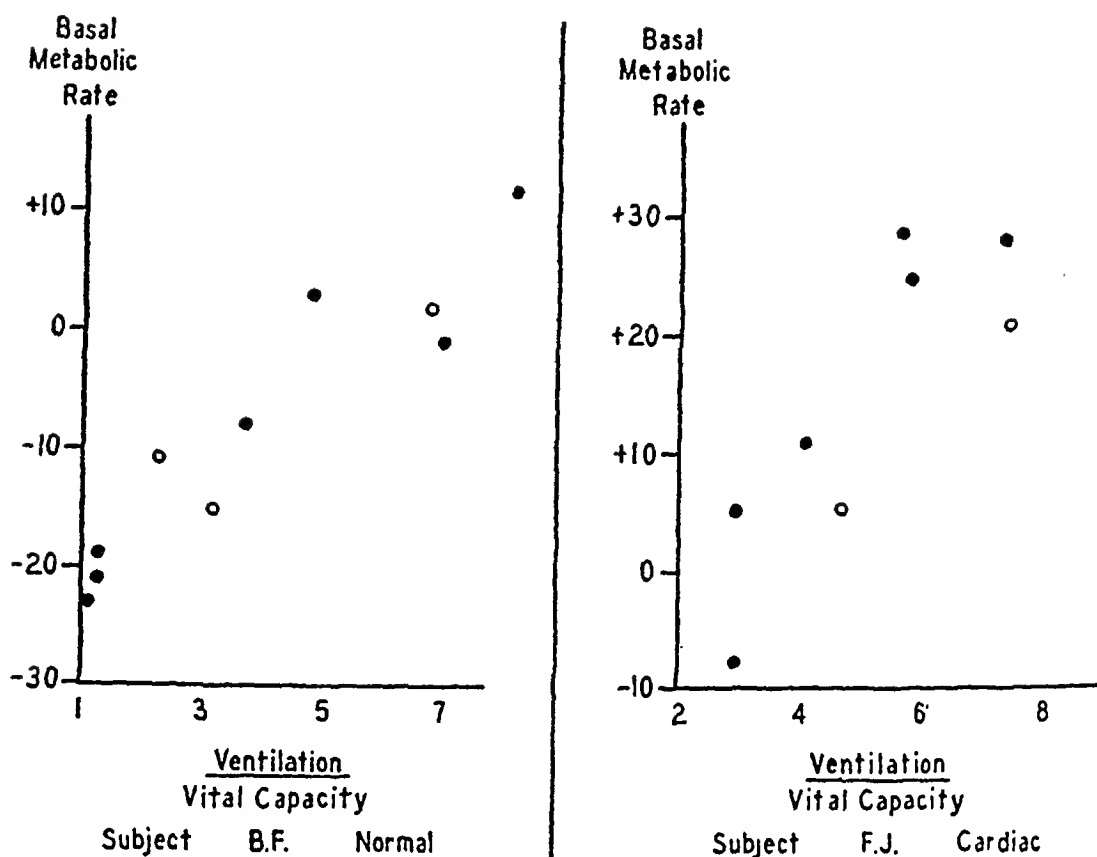


FIG. 6. CHANGES IN METABOLISM WITH OVERVENTILATION. ● VOLUNTARY  
○ CO<sub>2</sub>

The figure at the left represents the effects of changes in ventilation on the metabolic rate of a normal subject. The three black circles in the left lower corner are resting values. Both voluntary overventilation (black circles) and involuntary overventilation produced by carbon dioxide (hollow circles) resulted in a well marked increase in metabolic rate. Similar results as illustrated by the figure on the right were obtained in a patient with heart disease.

of hyperpnea varied in different individuals between 3 and 6 minutes, depending on the rapidity with which fatigue occurred. These variables are in part reflected in the wide scattering of the points. Nevertheless, there is an apparent correlation between the ratio  $\frac{\text{Ventilation}}{\text{Vital capacity}}$  and the metabolic rate. The elevations in metabolism due to voluntary overventilation are comparable to, though in general a little lower than those due to spontaneous dyspnea (Fig. 1).

The data in two typical experiments comparing normal respiration, voluntary hyperpnea and carbon dioxide overventilation are shown graphically in Figure 6. All observations were made at one sitting with 15 to 30 minute intervals between determinations. For a comparable increase in ventilation the elevation in metabolic rate seems more marked with voluntary than with involuntary hyperpnea. This is in agreement with the findings of Liljestrand cited by Loewy (6).

The most marked effect with carbon dioxide overventilation was shown by F. B. (Table III).

TABLE III

*The effect of carbon dioxide overventilation on the oxygen consumption*

(Subject F. B.: Syphilitic heart disease; vital capacity, 1750 cc.)

Period.....	1	2	3	4	5
Condition.....	Normal breathing	Normal breathing	Breathing 2 per cent CO <sub>2</sub>	Breathing 3 per cent CO <sub>2</sub>	Breathing 5 per cent CO <sub>2</sub>
Duration of breathing, minutes.....	6	6	4	3	2
Ventilation, liters per minute.....	8.4	8.1	11.7	24.7	24.3
Vital capacity.....	4.8	4.7	6.7	14.1	13.3
Oxygen consumption, cc. per minute.....	253	258	265	501	477
Pulse rate, per minute.....	90	88	104	104	104

Note an increase in metabolism of about 100 per cent in Period 4, and 90 per cent in Period 5.

The oxygen consumption almost doubled with a threefold increase in ventilation.

The effect of depressing the respiration with morphine was studied in six patients. In every dyspneic patient there was a well marked decline in ventilation and a slight or moderate decrease in metabolic rate, the magnitude of the change in both ventilation and metabolic rate varying with the initial level of  $\frac{\text{Ventilation}}{\text{Vital capacity}}$ . The results in two typical experiments are recorded in Table IV. Patient A. M., with a slightly elevated  $\frac{\text{Ventilation}}{\text{Vital capacity}}$  ratio of 2.9, showed no change in oxygen consumption beyond the limits of error of the method. Subject T. M. had marked dyspnea, an initial  $\frac{\text{Ventilation}}{\text{Vital capacity}}$  quotient of 10 and

an oxygen usage of 265 cc. per minute. Forty-five minutes after the subcutaneous injection of 0.02 gram of morphine the ratio had declined to 6.4 and the oxygen consumption to 202 cc. That this is not wholly due to the hypnotic effect of morphine is indicated by the fact that subsequently when the breathing of this patient was stimulated by carbon dioxide the oxygen consumption increased to 254 cc. per minute. Furthermore, non-dyspneic patients (as A. M.) showed very little or no change in metabolic rate following morphine.<sup>1</sup>

These observations demonstrate that the increased work of respiration in dyspneic patients is an important factor in the production of their elevated oxygen consumption.

#### *Oxygen consumption of the heart*

In evaluating the rôle played by the heart in the increased oxygen consumption in cardiac disease we are forced to depend largely on indirect evidence. The assumption that the heart plays such a rôle rests on two general propositions—first, that the failing heart is dilated; and second, that a dilated heart uses more oxygen in doing a given amount of work than a normal heart. The first has been amply demonstrated in the experimental animal by Patterson, Piper and Starling (8) and by Rohde (9), and in man by Stewart and Cohn (10). The second has been established by the work of Evans and Matsuoka (11) and Starling and Visscher (12). In the heart-lung preparation the latter authors found that the oxygen used by the heart is dependent on the diastolic volume, i.e., the degree of dilatation. As the heart tires it dilates and although the work remains constant the energy expended in doing it may be increased by as much as 60 per cent.

Harrison, Friedman and Resnik (13) studied the effect on the heart of the dog of certain injurious agents, namely, anoxemia, potassium chloride and chloroform. They obtained data for the calculation of the total metabolism, the cardiac oxygen consumption, and the cardiac work. As

<sup>1</sup> The observations on the effect of morphine on the ventilation suggest the possibility that in a patient with congestive failure and suspected hyperthyroidism because of increased oxygen consumption, the response to morphine may offer a means of determining the relative importance of thyroid and respiratory activity.

TABLE IV

#### *Effect of morphine on oxygen consumption and $\frac{\text{Ventilation}}{\text{Vital capacity}}$*

(A. Subject A. M.: Luetic heart disease, well compensated, showing very slight change in metabolism as result of a 28 per cent decrease in ventilation when the initial  $\frac{\text{Ventilation}}{\text{Vital capacity}}$  is slightly elevated.)

Condition	Normal breathing	45 minutes after morphine	1 hour after morphine	1½ hours after morphine. Breathing 3 per cent CO <sub>2</sub>
Oxygen consumption, cc. per minute.....	244	242	236	262
$\frac{\text{Ventilation}}{\text{Vital capacity}}$ ..	2.9	2.1	2.0	3.9

(B. Subject T. M.: Hypertensive heart disease—severe dyspnea, showing marked reduction in metabolism with 36 per cent reduction in ventilation when the initial  $\frac{\text{Ventilation}}{\text{Vital capacity}}$  is markedly elevated.)

Condition	Normal breathing	45 minutes after morphine	1 hour after morphine. Breathing 3 per cent CO <sub>2</sub>
Oxygen consumption, cc. per minute.....	265	202	254
$\frac{\text{Ventilation}}{\text{Vital capacity}}$ ..	10.0	6.4	7.7

cardiac failure occurred there was observed in all experiments an increase in the amount of oxygen used by the heart. This was noted even in the presence of a marked diminution in total oxygen consumption and in total cardiac work.

On the basis of these investigations there is little doubt that the heart does contribute to the elevated metabolism in cardiac disease. The question is whether its share is large or small. Starling and Evans (14), in the heart-lung preparation, found the oxygen consumption of the dog's heart to average 3.2 cc. per gram per hour. Comparable values were obtained by Harrison, Friedman and Resnik (13), in dogs with the chest and pericardium open. The procedure they employed causes considerable surgical shock. Because the lowered blood pressure and decreased cardiac output in shock results in diminished cardiac work, the data so obtained are not strictly applicable to animals in good condition. Using a new device these latter observers were able to determine the metabolism of the heart in animals with the chest intact. The average value for the cardiac oxygen consumption of morphinized dogs was found to be 5.3 cc. per gram per hour and about 11 per cent of the oxygen consumption of the whole animal. When the heart was made to overwork either by increasing the cardiac output (infusion of saline or blood) or by elevating the blood pressure (adrenalin, cutting the vagi, clamping the carotid arteries) its oxygen consumption per gram of muscle increased as much as 100 per cent. Under periods of maximum stress the oxygen consumption of the heart amounted to as much as 30 per cent of the total metabolism.

Clinically however, no such changes in cardiac work occur in congestive failure, for neither the cardiac output (15) nor the blood pressure vary very widely, nor in any consistent direction. While the total work of the heart does not change much, the energy expended in accomplishing it is greatly increased if the observations in the experimental animal with dilated and failing hearts are applicable to man. That this increased cost of performing work may be significant can best be illustrated by a concrete example. Let us make three assumptions:

1. The average oxygen consumption of the normal human heart is, like that of the dog's heart, 0.09 cc. per gram per minute.

2. The hypertrophied heart uses as much oxygen per gram as does the normal.

3. As the heart dilates and fails its oxygen consumption increases about 40 per cent. (This corresponds with the changes observed by Starling and Visscher in the heart-lung preparation, and by Harrison, et al., in the intact dog.)

If under these assumed conditions a patient with a normal oxygen consumption of 200 cc. per minute and with a heart weighing 250 grams, should develop cardiac hypertrophy (heart weight 500 grams), and later decompensation, then the total oxygen consumption, and that of the heart might be as shown in Table V.

TABLE V

*Oxygen consumption in a hypothetical case*

Condition of heart	Dyspnea	Heart weight	O <sub>2</sub> consumption of heart	O <sub>2</sub> consumption of the rest of the body	Total O <sub>2</sub> consumption	Basal metabolic rate
		grams	cc. per minute	cc. per minute	cc. per minute	per cent
Normal.....	Absent	250	22.5	177.5	200	± 0
Compensated, hypertrophied..	Absent	500	45	177.5	222.5	+11
Decompensated, hypertrophied..	Absent	500	63	177.5	240.5	+20
Decompensated, hypertrophied..	Present	500	63	227.5*	290.5	+45

\* Including effect of dyspnea. An increase of 25 per cent in oxygen consumption due to respiratory effort in severe dyspnea  $\left[ \frac{\text{Ventilation}}{\text{Vital capacity}} \text{ ratio} = 6-8 \right]$  is consistent with the data represented in Figure 5.

In Table V, which may be taken as representative of severe congestive failure, the increased cardiac oxygen consumption accounts for an increment of 20 per cent in the total metabolism.

#### SUMMARY

The effect of therapy upon the oxygen consumption of twenty-five patients with heart disease was observed. It was noted that in general those with the most marked evidence of congestive failure had the greatest elevation of metabolic rate, and showed the most pronounced decline in oxygen consumption with improvement. There was a rather striking parallelism between changes in the degree of dyspnea as measured by the ratio

$\frac{\text{Ventilation}}{\text{Vital capacity}}$  and the variations in the metabolic rate. The effect upon the oxygen consumption of increased ventilation produced both voluntarily and by the inhalation of carbon dioxide was measured in both normal subjects and in patients with heart disease. In general, it was found that an increase of the ratio  $\frac{\text{Ventilation}}{\text{Vital capacity}}$  was associated with a rise in the oxygen consumption of sufficient magnitude to account for a large portion of the elevation in metabolic rate found in patients with cardiac dyspnea. Measures, such as venesection, paracentesis and the administration of morphine, which lower the ratio  $\frac{\text{Ventilation}}{\text{Vital capacity}}$ , were usually effective in reducing the oxygen consumption of dyspneic patients. In one subject the re-infusion of blood that had been removed a short time previously caused the ventilation and oxygen consumption to rise to about their pre-existing values.

In addition to the effect of labored breathing, if it can be assumed that observations made upon the failing heart of the dog can be applied to the diseased heart of man, then calculations indicate that the oxygen consumption of the heart also may be an important factor in the elevation of the basal metabolic rate occurring in patients with congestive failure.

#### DISCUSSION

The above observations indicate that the increased work of the respiratory muscles associated with cardiac dyspnea adds another load to an already overburdened heart. This factor of respiratory effort assumes greater importance as cardiac failure becomes worse, for the ventilation tends to rise and the vital capacity to fall, thus increasing the ratio  $\frac{\text{Ventilation}}{\text{Vital capacity}}$  in geometric proportions. Procedures which tend to reduce the ventilation (rest, morphine, venesection, paracentesis) or to increase the vital capacity (paracentesis) are beneficial in part through reduction of the work of breathing.

In advanced congestive failure with severe dyspnea the factor of labored breathing seems to play the most important rôle in elevating the me-

tabolism. The increased oxygen consumption of the diseased heart ranks next in importance. Other factors appear to be relatively minor. Of course, in evaluating the significance of an elevated basal metabolic rate in a given patient with cardiac disease the factors of fever, nervousness and thyroid activity must be considered as in any other patient.

#### CONCLUSIONS

1. The basal metabolic rate is elevated in many persons with congestive cardiac failure and declines as improvement occurs. The degree of elevation (and decline) tends to parallel the severity of congestive failure (and the extent of improvement).
2. The mechanisms responsible for these changes have been investigated. The increased work of the muscles of respiration appears to be the chief factor; the diminished mechanical efficiency and consequent increased energy expenditure of the diseased heart may also play an important rôle.

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## STUDIES OF KIDNEY FUNCTION IN CHILDREN

### I. UREA CLEARANCE VALUES: (1) NO EVIDENCE OF KIDNEY DISEASE (2) AFTER ACUTE HEMATURIC NEPHRITIS FOLLOWING AN ACUTE INFECTION (3) IN THE ACUTE STAGE OF HEMATURIC NEPHRITIS<sup>1</sup>

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This study was made with three distinct purposes in view. The first was to determine whether the range and distribution of urea clearance values in children coincided with those in adults. The principles of the urea clearance test of renal function and its usefulness for adults have been adequately established by Möller, McIntosh and Van Slyke (1), and by other workers. There has been some question whether this test is as adequate for children as for adults.

The second purpose was to determine if it were possible by the urea clearance test to detect residual damage in the kidneys of children who had previously had acute hematuric nephritis. The general consensus of opinion is that, in the great majority of such cases, recovery of the kidney is complete. However, the urea clearance test has not been utilized in any extensive study of this problem.

The third purpose was to use the urea clearance test in children during the acute stage of hematuric nephritis, as an index of the degree of kidney damage, and to ascertain its usefulness during the convalescent stage in determining the rate at which the kidneys return to normal function.

#### DETAILS OF THE EXAMINATIONS

*The control group.* The 62 children in this group who were residents at the Children's Convalescent Home, had no evidence of kidney damage and no history of kidney disease. The groups included children who were primarily nutritional problems, children with some type of chronic heart disease, and children convalescing from various other conditions. This group of 62 chil-

dren not only served as the control for the group who had a history of acute hematuric nephritis but also furnished data concerning the urea clearance values to be expected in abnormal conditions other than kidney disease.

The children were given a breakfast of fruit, cereal with milk and an additional glass of milk. They received no eggs, meat or coffee. Approximately an hour after breakfast they voided and drank one glass of water. At the end of one hour they again voided and this urine was collected. Following this a sample of blood was taken from a vein, and a second glass of water given. At the end of the second hour the final sample of urine was collected. Complete collection of the samples was insured. While each collection period was not exactly an hour, the time at which the sample was taken was carefully recorded. This is the routine procedure recommended by Möller, McIntosh and Van Slyke (1). In all the data presented here the term "test" indicates the average of these two successive clearance values. It is to be noted that, with few exceptions, blood was taken following the collection of the first sample of urine.

The urea determinations on both the blood and urine were made with the Van Slyke manometric apparatus. 0.2 cc. of blood being used. Most determinations were done in duplicate. All the clearance values were corrected on the basis of the height of the child by the factor from McIntosh, Möller and Van Slyke's (2) line diagram.

Certain details of the urea determination should be noted. It is not possible with the gasometric urease method to get satisfactory blank determinations on 0.2 cc. of pure urea solutions corresponding to blood urea concentrations. The values are always low. However, with blood, the 0.2 cc. technic checks satisfactorily against the aeration technic, using 3 cc. for the same blood. Without giving in detail the studies which were

<sup>1</sup> Presented in abstract before the Central Society for Clinical Research, Chicago, November 1934.

made in an attempt to explain this fact, our conclusion was that in the absence of proteins of the blood the minute amount of mercury dissolved inhibits urease action whereas with protein present the slight amount of mercury that may be dissolved combines with the protein and therefore does not effect the enzyme. A minor deviation from Van Slyke's directions consisted in allowing the urease solution to act on the blood for at least two minutes, in order to insure complete transformation of the urea into ammonium carbonate. However, the possibility of significant ammonia formation from other nitrogen constituents of the blood was obviated by never allowing the urease to act more than 4 minutes. With this method, blanks on the urease solution should be run practically each day. "Double strength" (Squibb) urease powder should be used. Urease powder that has aged for several months is more satisfactory than fresh powder since it gives a lower and more constant "blank" value.

*The group with a history of acute hematuric nephritis.* Children who had had hematuric nephritis following an acute infection were brought to the clinic of the Children's Hospital for examination.<sup>2</sup> To avoid the factor of selection in this group an attempt was made to examine all children who had had acute nephritis within the past ten years and had been patients at the Children's Hospital or the Cincinnati General Hospital. For obvious reasons many of these children could not be returned for examination. However, it is probable that parents of children, who had had any persistent illnesses following their hospitalization, would be more willing to have them returned, and it is likely, therefore, that this group would include the greater proportion of children with permanently damaged kidneys, if such damage existed. Instructions were given that the children should receive the same breakfast outlined for the control group, and we believe that this procedure was followed in most instances. The children reported to the Clinic approximately an hour after breakfast. Clearance tests were then performed as in the control group. An interim history was taken, and a complete examination was made, which included blood pressure readings and an analysis of the urine for albumin, sugar, specific gravity, pH and microscopic study. In most instances the children appeared to be undisturbed by the examinations.

<sup>2</sup> In this task we had the efficient cooperation of the Social Service Department of the Children's Hospital.

#### RECORDING AND ANALYSIS OF DATA

A large amount of accessory data were accumulated which cannot be reported here. Unless an exception is stated either in the tables or in the discussion, the physical and urine examinations and the history indicate that the child's condition was essentially normal.

The recording of data relating to children with a history of acute hematuric nephritis requires some explanation. In many cases, either because of an apparently unusual difference between the two successive clearance values of one test or because of some question concerning the clinical condition, the child returned to the clinic one or more times. In only two cases did these repeated tests vary significantly from each other. One of these is Number 5 of Table IV, who apparently showed a delayed recovery. The other (Case 92) was a girl so extremely apprehensive that three determinations had to be discarded. On the fourth visit to the clinic the disturbing psychic factors were eliminated, and the examination completed. In addition, several of the children first studied have been brought back after one or two years for further examinations.

The following arbitrary selection of data has been made. In Table II the last test made on the child is recorded. The histogram for this group (Figure 1) showing the frequency of occurrence of urea clearance test values is, therefore, based on the number of children rather than the number of determinations.

The number of children is sufficient to allow a biometric analysis of several secondary questions, such as the relation of clearance to age, to sex and to the time elapsed since the acute condition. For these analyses the repeated tests on any given child are actually new data and are so used. For example, a child who had acute hematuric nephritis at 10¼ years was studied two years ago at the age of 11 years. He has been studied twice since at 11½ and 12½ years of age respectively. The last determination at 12½ years is used for Table II and Figure 1. The first analysis is used for the analysis of "time since cessation of acute symptoms" in the "1 month to 1 year" group and the average of the last two determinations for the corresponding group "1 to 5 years." For the analysis of the influence of age, the first two



TABLE II  
*Urea clearance values in children who had acute hematuric nephritis following an acute infection*

Subject number	Age	Sex	Height	Weight	T.S.A.S.*	Clearance (C) data									
						Blood urea	1st period				2nd period				C average
							Urine			C <sub>1</sub>	Urine			C <sub>2</sub>	
							Urea	V	V <sub>cor.</sub>		Urea	V	V <sub>cor.</sub>		
	years		inches	pounds	years, months	mgm. per 100 cc.	mgm. per 100 cc.	cc. per minute	cc. per minute	per cent normal	mgm. per 100 cc.	cc. per minute	cc. per minute	per cent normal	per cent normal
104-2	18	F	59	90	3-5	25.8	258	3.51	4.42	59	185	5.03	6.33	61	60
187	7	M	44.5	45	2-7	31.5	1378	0.54	1.15	87	241	2.97	6.30	64	76
188	10	F	48	58	3-4	21.4	362	1.90	3.50	79	184	3.62	6.66	76	78
80-2	18	F	62.5	90	7-0	17.9	245	3.90	4.52	84	186	4.47	5.18	72	78
101	8	F	50	50	1-0	12.5	114	3.79	6.59	80	239	1.72	2.99	76	78
88-3	12	F	64	100	1-10	20.0	790	1.32	1.49	89	418	2.43	2.75	76	83
103	10	M	56	64	-11	27.1	205	5.71	8.34	84	335	3.57	5.21	85	85
90-2	13	F	52	69	3-0	20.4	459	1.82	2.95	88	231	3.45	5.59	84	86
149	18	M	65	116	6-0	29.0	900	2.63	2.84	118	442	2.52	2.72	55	87
97	11	F	54	58	1-6	16.9	298	2.58	3.90	92	204	3.45	5.21	84	88
76	7	F	47	53	4-0	23.9	585	1.53	2.94	96	768	0.98	1.88	81	89
186	13	M	61	111	2-6	26.6	620	2.42	3.00	93	644	2.22	2.75	89	91
176	14	M	59.5	88	5-2	20.2	372	2.77	3.63	89	199	5.45	7.14	93	91
82	11	M	58	97	6-0	26.6	935	1.51	2.10	99	325	3.75	5.21	85	92
183	11	F	51	54	4-4	22.6	276	3.63	6.13	100	164	5.08	8.59	83	92
174	9	F	50	52	1-2	20.7	250	3.40	5.92	95	186	4.25	7.40	89	92
59-2	10	M	53	57	-7	30.2	360	3.96	6.26	100	633	1.93	3.05	85	93
112	9	M	53	65	3-8	18.9	529	1.58	2.53	95	458	1.78	2.85	92	94
196	10	F	50	60	3-8	19.9	625	1.47	2.54	107	190	3.67	6.35	81	94
75-6	9	M	53	68	1-0	20.1	215	4.42	6.94	99	200	4.22	6.63	88	94
92-4	12	F	57	96	2-0	34.6	753	2.33	3.26	95	329	5.42	7.59	96	96
189	8	F	49.5	59	3-6	21.7	284	3.47	6.14	107	158	4.98	8.81	85	96
94	9	M	48	54	1-4	24.0	428	2.41	4.53	108	290	2.88	5.41	87	98
83	9	F	51	54	4-8	25.1	472	2.57	4.37	109	391	2.45	4.17	87	98
102	9	M	50	50	1-3	23.3	303	3.23	5.68	99	238	4.17	7.34	100	100
151-2	14	M	56.5	86	3-0	21.5	614	1.73	2.47	94	217	5.47	7.82	105	100
179-2	14	M	67.5	129	6-8	31.2	1694	0.93	0.96	98	1260	1.78	1.83	102	100
177	13	M	56	75	4-6	18.5	200	4.52	6.55	95	230	4.45	6.45	107	101
185	18	M	70.5	144	4-0	19.5	505	3.37	3.17	109	656	2.18	20.5	92	101
89	13	F	58.5	81	5-0	15.9	348	2.66	3.59	105	196	4.40	5.94	97	101
99	9	F	51	63	1-1	29.4	738	2.10	3.55	119	400	2.78	4.70	85	102
4-2	6	M	40.5	43	-7	22.8	229	3.50	7.74	104	164	4.72	10.43	100	102
63	12	M	55.5	74	2-3	25.2	882	1.38	2.03	95	690	2.07	3.04	111	103
72-3	9	M	47	46	1-3	24.2	391	2.67	5.18	112	544	1.60	3.10	93	103
69-3	13	M	66	125	5-6	25.8	480	3.65	3.87	96	312	6.50	6.89	111	104
100	4	M	41	39	2-7	16.6	126	5.00	11.85	120	185	2.62	6.21	92	106
194	12	F	56	84	4-0	20.1	328	3.18	4.64	101	212	5.40	7.88	111	106
191	11	F	55	95	4-8	15.1	870	0.93	1.39	126	293	2.28	3.42	88	107
73	12	M	59	100	3-0	32.0	1582	1.29	1.70	120	595	2.87	3.79	94	107
3	9	M	50	62	-1	23.4	1993	0.23	0.40	100	1672	0.45	0.78	116	108
111	11	F	52	59	3-0	21.9	728	1.57	2.54	113	293	3.57	5.78	103	108
98	5	M	41.5	36	1-2	14.0	275	1.94	4.52	119	100	4.48	10.44	99	109
95	16	M	68	143	5-0	22.0	430	4.27	4.38	114	1092	1.21	1.24	103	109
5-3	14	F	60.5	107	1-8	24.9	1345	1.11	1.41	119	375	3.93	4.99	100	110
165	9	M	50	57	1-8	25.1	1364	0.81	1.40	119	190	5.73	9.97	100	110
110	8	F	48	50	2-11	15.7	605	1.20	2.26	116	162	4.00	7.52	104	110
67	10	F	51	60	2-4	25.0	813	1.67	2.82	122	230	4.77	8.06	99	111
74-9	7	M	51	65	-1.5	27.3	573	2.38	4.24	119	242	5.00	8.90	105	112
107	4	F	40.5	35	-11	28.0	2005	0.43	1.03	135	464	1.73	4.15	92	114
85-2	15	F	63	99	1-3	30.5	3359	0.34	0.40	129	1252	1.40	1.65	98	114

\* Time since acute symptoms.

TABLE II (continued)

Subject number	Age	Sex	Height	Weight	T.S.A.S.*	Clearance (C) data									
						Blood urea	1st period				2nd period				C average
							Urine			C <sub>1</sub>	Urine			C <sub>2</sub>	
							Urea	V	V <sub>cor.</sub>		Urea	V	V <sub>cor.</sub>		
	years		inches	pounds	years, months	mgm. per 100 cc.	mgm. per 100 cc.	cc. per minute	cc. per minute	per cent normal	mgm. per 100 cc.	cc. per minute	cc. per minute	per cent normal	per cent normal
91	4	F	39	34	1-8	23.9	2434	0.26	0.65	152	749	0.71	1.78	78	115
121-2	8	M	48.5	56	-2	20.8	340	2.62	4.82	105	243	4.42	8.13	127	116
71	8	F	47	51	2-7	21.3	1790	0.33	0.64	124	444	1.97	3.84	107	116
108	12	F	58	75	5-4	21.6	278	5.55	7.60	130	301	3.98	5.45	101	116
105-2	13	M	54	70	2-2	17.4	570	1.77	2.73	119	269	3.57	5.50	113	116
190	7	M	42.5	43	3-3	17.7	160	5.03	11.32	136	98	5.80	13.05	96	116
166	9	F	50	58	1-3	19.2	355	2.82	4.91	121	182	5.10	8.87	112	117
113-3	12	F	62	95	3-6	15.4	202	5.73	6.99	122	245	4.35	5.31	113	118
64	7	M	45	46	1-8	25.9	1154	0.95	2.00	119	180	6.22	13.06	121	120
169	9	F	50	50	3-0	16.8	456	1.93	3.36	122	166	5.20	9.05	119	121
93	11	M	60.5	98	1-6	19.2	1532	0.55	0.70	124	1270	0.75	0.95	119	122
181	17	M	68.5	142	9-6	24.5	1574	1.21	1.20	130	1389	1.17	1.16	113	122
197	14	M	63	99	8-8	16.6	1663	0.49	0.57	140	626	1.80	2.09	105	123
168	5	F	42.5	40	3-0	16.7	400	1.52	3.91	125	239	2.55	6.55	125	125
66	5	F	44	47	1-0	23.7	232	4.65	10.14	132	254	3.85	8.39	120	126
184	19	M	69	129	11-0	28.4	2987	0.52	0.50	138	1747	1.02	0.99	113	126
2-3	11	M	58	88	1-6	23.0	334	5.10	6.99	136	397	3.73	5.11	117	127
195	7	M	44.5	42	1-4	26.0	3452	0.20	0.43	161	472	1.83	3.97	96	129
173	15	M	64.5	147	3-0	27.9	2070	0.88	0.99	137	588	3.94	4.40	124	131
81	12	M	56	71	5-0	34.3	3618	0.38	0.54	144	2816	0.42	0.61	119	132
65	15	M	60	93	3-4	19.1	477	3.40	4.39	146	240	5.50	7.10	119	133
86	14	M	62	91	4-8	27.9	2457	0.71	0.87	152	1126	1.80	2.20	119	136
109	6	F	44	44	5-4	17.3	1711	0.34	0.73	157	338	2.12	4.56	119	138
79	12	M	54	78	4-0	26.2	1936	0.80	1.24	152	526	3.02	4.68	125	139
106	11	M	55	78	4-7	24.1	2373	0.51	0.76	159	318	4.56	6.84	120	140
180	10	M	51	67	5-0	22.4	566	3.32	5.58	188	287	4.17	7.01	120	154
192	13	M	56	73	4-6	29.0	2703	0.59	0.87	161	2800	0.51	0.75	155	158
150	7	M	48	51	2-0	32.7	610	4.58	8.47	211	547	2.95	5.46	122	167

not similar to ours since they apparently reported the incidence of determinations rather than, as we did, the number of children examined. They also gave urea preliminary to the test in order to insure maximum clearance. However, their results are in agreement with our conclusions.

Holt (4) called attention to the greater variability of the augmentation limits in children and suggested that this might be a serious limitation to the usefulness of the test in children. The present data indicate that, for children over 4 years, this suggested limitation does not hold. We have not studied children under 4 years of age but Schoenthal, Lurie and Kelly (5) reported that in 9 normal infants urea clearance values corresponded to those of normal adults.

It would appear, therefore, that the blood urea

clearance test of renal function is as applicable to the study of renal damage in children as it is in adults and that the range and distribution of values are similar.

*The group with a history of acute hematuric nephritis.* The data for these children are given in Table II and summarized to show the distribution of clearance values in the lower histogram of Figure 1.

It is evident at once that the second purpose of this study, namely, to determine whether there is any evidence of consistently occurring residual damage that is detectable by the clearance test in children with a history of hematuric nephritis, is answered in an unequivocal manner. That is to say, the mean and distribution of the test values of this group are indistinguishable from those of

the control group. (See Figure 1.) It is especially significant that the value for standard deviation of this group, like that of the control group, indicates a "stable universe."

While it seems clear that in this group as a whole the *ability of the kidneys to excrete urea is essentially normal* there were several children who had moderately low clearance values and who merit special consideration. See below under "Clinical Discussion."

that one month may be arbitrarily selected as the time when the immediate effect of the acute stage had ceased. The data were accordingly divided on the basis of intervals since cessation of acute symptoms of 1 month to 1 year; 1 to 5 years; and 5 years or more. It appears (Table III) that there is no significant variation between these sub-groups. The mean for the sub-group "1 month to 1 year" of 104.2 is somewhat less than the mean for the whole group but the difference

TABLE III  
*Biometric analysis of urea clearance tests*

History of acute hematuric nephritis				No evidence of kidney damage		
	Sub- jects	Mean C	Standard deviation	Sub- jects	Mean C	Standard deviation
Entire group	78	<i>per cent normal</i> 109.1 $\pm$ 1.5	19.2 $\pm$ 1.0	62	<i>per cent normal</i> 107.3 $\pm$ 1.4	15.9 $\pm$ 1.0
Age, years						
4-8	16	116.9 $\pm$ 3.3	19.7 $\pm$ 2.3	14	107.9 $\pm$ 2.4	13.2 $\pm$ 1.7
8-12	33	106.1 $\pm$ 2.0	17.3 $\pm$ 1.4	35	106.2 $\pm$ 1.7	15.1 $\pm$ 1.2
12-16	24	111.5 $\pm$ 2.4	17.3 $\pm$ 1.7	13	109.4 $\pm$ 3.7	20.0 $\pm$ 2.6
16+	6	96.7 $\pm$ 6.1	22.2 $\pm$ 4.3			
Boys	45	113.5 $\pm$ 2.0	19.6 $\pm$ 1.4	42	108.1 $\pm$ 1.7	16.5 $\pm$ 1.2
Girls	33	103.1 $\pm$ 2.0	17.0 $\pm$ 1.4	20	105.5 $\pm$ 2.2	14.4 $\pm$ 1.5
CmCm	52	104.7 $\pm$ 1.7	18.2 $\pm$ 1.2	47	107.5 $\pm$ 1.5	15.7 $\pm$ 1.1
CsCm	15	117.8 $\pm$ 3.3	18.7 $\pm$ 2.3	14	106.1 $\pm$ 3.0	16.5 $\pm$ 2.1
CsCs	9	122.5 $\pm$ 3.4	15.3 $\pm$ 2.4			
Cm				47	112.1 $\pm$ 2.0	19.9 $\pm$ 1.4
Cs				15	116.2 $\pm$ 2.5	14.4 $\pm$ 1.8
T.S.A.S.*						
1-12 months	12	104.2 $\pm$ 3.1	16.0 $\pm$ 2.2			
1-5 years	57	108.3 $\pm$ 1.8	20.3 $\pm$ 1.3			
5+	15	112.5 $\pm$ 3.5	19.9 $\pm$ 2.5			
Activity and cardiac lesions						
Bed (includes 6 cardiacs)				12	105.0 $\pm$ 3.7	19.0 $\pm$ 2.6
Bath and dining (includes 1 cardiac)				12	113.8 $\pm$ 2.8	14.3 $\pm$ 2.0
All heart cases				13	111.3 $\pm$ 3.0	15.8 $\pm$ 2.1
Limited and full (includes 6 cardiacs)				38	105.9 $\pm$ 1.6	14.8 $\pm$ 1.1
Limited and full (No cardiacs)				32	105.8 $\pm$ 1.9	16.0 $\pm$ 1.3

\* Time since acute symptoms.

Although the distribution histogram of this data, which parallels that of the control group, suggests that analysis of sub-groups could show only minor differences, some of the analyses are interesting because of the very lack of influence of factors often considered important.

One of the first questions was that of the influence of the time interval since cessation of acute symptoms (Table II). From the data of the "acute group," Table V, it may be concluded

is not significant. It would, however, be definitely incorrect to conclude that all traces of acute damage have always entirely disappeared within one month after cessation of the acute symptoms. Case 5, for example, showed at three months a definitely lowered clearance which became normal after 3½ months.

At first glance there would appear to be a somewhat greater influence of age on the clearance in this post-nephritic group than in the con-

rol group, but more careful study shows that here is no mathematically significant difference between the mean for the age sub-groups from four to sixteen years. The number of children in the sub-group "16 years or more" is so small (6) that no significance can be attributed to its low value.

The difference between mean values for boys and for girls might suggest a sex factor for this group. However, the low mean value for the girls is not significantly different from the means of the total of either major group. Moreover the difference between the mean values of the boys and of the girls is not significant by biometric criteria. That is to say, in order for the difference of the two means to be significant the value of the difference of the means divided by the probable error of the difference

$$\left( K = \frac{\text{Difference}}{\text{Probable error difference}} \right)$$

should be greater than four (odds 142 to 1), whereas the difference between the means of boys and girls in this instance is only 3.71. Stating this in another way, the odds are only 80 to 1 that this difference between the means of boys and girls is not due to chance alone.

It is an interesting coincidence that the coefficients of variation of urea clearance of our cases in the two major groups are about 17 which is close to the coefficient of variation of the size of the healthy kidney, 16.8 (6). The authors do not take this to mean that variations in urea clearance are wholly dependent on variation in size of kidneys.

In both groups of children phenolsulphonphthalein tests were made coincidently with the clearance tests. The comparison of the two tests for each child showed so little correlation that it does not seem worth while to tabulate them. This lack of correlation may be due to the fact that the dye

TABLE IV

*Additional data on those children of the group with a history of hematuric nephritis who had either a relatively low clearance or other symptoms of kidney abnormality*

Subject number	Age	Sex	T.S.A.S.†	Blood pressure	Urine	Blood urea nitrogen	Urea clearance			Phenolsulphonphthalein'			Concentration test
							1st period	2nd period	Average	1st hour	2nd hour	Total	
104	years 18	F	years-months 2 3 3 5	mm. Hg 106/76 124/84	0 alb. +	mgm. per 100 cc. 10 12	per cent 55 59	per cent 56 61	per cent 56 60	per cent 39 34	per cent 22 29	per cent 61 63	specific gravity
187	7	M	2 7	98/66	0	15	87	64	76	53	13	66	
80	18	F	6 7	118/78 114/78	0 0	11 8	79 84	73 72	76 78	46 53	41 19	87 72	
188	10	F	3 4	110/68	0	10	79	76	78	31	28	59	
101	8	F	1	108/70	Tr. alb.	6	80	76	78	23	23	46	
88	12	F	1 6 1 10	120/90	alb. ++	6 10 9	73 78 89	71 79 76	72 79 83	39 56 64	24 22 9	63 78 73	1.027*
179	14	M	6 4 6 8	130/74 111/68	alb. + 0	13 15	141 98	100 102	121 100	34 67	28 10	62 77	1.031*
69	13	M	4 9 4 5 5 6	158/100 130/84 120/90	alb. ++ alb. ++ alb. +	10 11 12	108 112 96	128 106 111	118 109 104	44 20 61	27 30 10	71 60 71	1.024⊕ 1.020*
5	14	F	3 3½ 1 8	110/80 110/70	0 0 alb. +	10 13 12	38 115 119	60 134 100	49 125 110				

† Time since acute symptoms.

‡ Corrected to hourly basis.

\* Lashmet and Newburgh, (9).

⊕ Mosenthal (10).



was injected subcutaneously, and the statement may not be true for results of intravenous phenolsulphonphthalein injections or of the fractional test (Shaw (7), Chapman and Halsted (8)).

*Clinical discussion.* Additional data are recorded in Table IV concerning six children who had clearance values below 85 (first six cases of Table II) or had evidence suggestive of kidney disease despite the normal clearance value. (See Cases 179, 69 and 5.)

Three of the children (Cases 80, 187 and 188) had no symptoms suggestive of kidney damage.

It should be noted that in five of these six children with relatively low clearance there was a comparatively small difference between the successive hourly clearance values (exception 187). This in itself is probably without significance but if associated with continuously low or decreasing clearance values, it may possibly be considered evidence of loss of flexibility of kidney function.

The urine of three children (Cases 104, 101 and 88) contained albumin. In one of these three (Case 88) the albuminuria was of the orthostatic type and the kidney was able to concentrate the urine to a specific gravity of 1.027.

The last three children listed in Table IV (179, 69 and 5) had clearance values well within the normal range but had albuminuria on at least one examination. In only one (Case 69) is there general evidence for a diagnosis of chronic nephritis. He had had a constant albuminuria with few or no casts in the urinary sediment. His systolic blood pressure ranged from 120 to 160; it has been as high as 140 since the last examination noted in Table IV. In addition his kidneys were unable to concentrate urine to a specific gravity of more than 1.020. While he had had no manifest edema, on several occasions there were fluctuations in weight of 6 to 8 pounds.

None of the children listed in this table is incapacitated. It is planned to follow the future progress of all of them.

#### DETERMINATION OF UREA CLEARANCE VALUES OF PATIENTS WITH ACUTE HEMATURIC NEPHRITIS

In Table V are given the data for the children who were studied during the acute stage of hematuric nephritis. All but two (Cases 152 and 161) were studied also during and after convalescence.

Five cases are included (5, 59, 121, 160, 164) for which there are no data during the acute stage.

The manner in which the kidneys recover from the acute attack is shown clearly in this table. It is apparent that, in general, the clearance value has usually returned to the normal zone within one month, although there are exceptions (see Cases 5 and 160). In Case 5 the clearance was only 49 three months after acute symptoms had ceased, but after four months the clearance was normal. In Case 74 there was a stormy course in the hospital with a second acute attack following rheumatic infection three months after admission to the hospital.

The data for this group demonstrate the usefulness of the clearance test for determining the extent of initial damage to the kidneys and for ascertaining when the function of the kidney has been restored to normal.

#### SUMMARY

1. The blood urea clearance values were determined in a group of 62 children who had no evidence of kidney disease. The results are analysed biometrically. The distribution and mean values corresponded to those of normal adults.

2. Similar studies were made in a group of 78 children with a history of acute hematuric nephritis. The distribution and means of the urea clearance values coincided with those from the group in which there was no history of kidney damage. There is evidence that most of these children had no residual kidney damage due to their acute condition.

3. Data are given for the clearance values during the acute stage of, and convalescence from, hematuric nephritis. In the majority of cases the kidney function had returned to normal within one month after cessation of the acute symptoms.

4. The two types of clearance values, "maximum" and "standard," appeared to be entirely comparable.

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TABLE V

*Urea clearance values in children during the acute stage of, and convalescence from, hematuric nephritis*

Subject number	Age	Sex	Height	Weight	Time since onset	T.S.A.S.†	Clearance (C) data										
							Blood urea	1st period				2nd period				C (av.)	
								Urine			C <sub>1</sub>	Urine			C <sub>2</sub>		
								Urea	V	V <sub>cor.</sub>		Urea	V	V <sub>cor.</sub>			
	years		inches	pounds			mgm. per 100 cc.	mgm. per 100 cc.	cc. per minute	cc. per minute	per cent normal	mgm. per 100 cc.	cc. per minute	cc. per minute	per cent normal	per cent	
74-1	7	M	51	58	13 da.	(acute)	216	711	0.09	0.15	2	25 hour specimen				2	2
2			51	61.5	18 "	"	111.8	608	1.64	2.77	20	456	2.10	3.55	19	20	
3				69.5	20 "	"	54.7	711	0.20	0.34	14	617	1.37	2.32	35	25	
4				61	23 "	"	39.1	811	0.66	1.12	41	639	1.04	1.76	40	41	
5				58	26 "	3 da.	28.3	795	0.70	1.18	57	611	1.39	2.35	68	63	
6				56	31 "	8 "	30.4	821	1.08	1.83	68	731	1.50	2.54	80	74	
7				55	40 "	17 "	31.2	1127	0.32	0.53	49	903	1.40	2.37	92	71	
8				56	51 "	28 "	26.2	1829	0.37	0.62	102	784	2.14	3.62	144	123	
9			51		67 "	44 "	27.3	573	2.38	4.24	119	242	5.00	8.90	105	112	
10			51		31 "	28 "	28.7	665	2.67	4.51	139	737	2.25	3.78	129	134	
75-1	8	M	51.5		18 da.	(acute)	19.9	270	1.60	2.67	48	220	2.60	4.34	64	56	
2					21 "	2 da.	27.1	541	1.23	2.05	55	268	2.44	4.07	54	55	
3					26 "	7 "	23.9	653	1.31	2.19	80	538	1.44	2.40	72	76	
4			52	58	35 "	16 "	25.0	335	2.40	3.98	71	286	3.55	5.89	90	81	
5					47 "	28 "	24.6	1810	0.63	1.05	139	238	3.22	5.35	69	104	
6	9		53		13 mo.	12 mo.	20.1	215	4.42	6.94	99	200	4.22	6.63	88	94	
162-1	8	F	54		5 da.	(acute)	120.1	1050	0.39	0.59	13	1201	0.43	0.66	15	14	
2					11 "	"	28.5	709	0.61	0.94	44	253	2.60	4.00	47	46	
3					18 "	"	51	873	0.33	0.51	23	715	1.42	2.19	41	32	
4					25 "	6 da.	41.7	1621	0.46	0.71	61	557	2.42	3.73	67	64	
200-1	8	F	50	48	2 da.	(acute)	54.5	401	1.75	3.05	30	339	1.63	2.84	22	24	
2				46	7 "	"	38.5	243	3.73	6.49	55	227	3.62	6.30	50	53	
3				48	12 "	3 da.	38.1	317	2.92	5.08	56	203	4.32	7.52	53	55	
4				47	21 "	12 "	24.9	253	3.55	6.18	84	161	4.95	8.61	75	80	
134-1	14	M		107	8 da.	(acute)	32.8	1075	0.43	0.49	42	1192	0.62	0.71	57	50	
2			63.5	98	14 "	"	44.5	986	1.35	1.55	51	967	1.50	1.73	53	52	
3				97	22 "	7 da.	29.5	1290	1.18	1.36	94	760	2.37	2.73	94	94	
159	12	M	59	78	10 da.	(acute)	23.6	246	2.63	3.50	49	252	2.57	3.42	49	49	
198-1	9	F	53	54	13 da.	(acute)	43	1388	0.35	0.55	44	693	1.97	3.09	66	55	
2			53.5	67	5 mo.	4½ mo.	30.8	3005	0.37	0.59	139	1012	1.24	1.95	85	112	
161	7	F	47	42	13 da.	(acute)	27.6	1162	0.19	0.38	48	530	0.73	1.44	43	46	
152	5	M	41		14 da.	(acute)	37.1					140	3.55	8.38	42	42	
170-1	5	M	45.5		14 da.	(acute)	31.5	217	1.12	2.28	21	180	0.49	1.01	11	16	
2					21 "	1 da.	25	1286	0.41	0.84	87	1277	0.35	0.72	80	84	
114-1	7.5	F		43	24 da.	(acute)	47	490	3.50	5.92	82	396	2.83	4.78	53	68	
2			50.5	46	63 "	35-40 da.	30.5	1609	0.62	1.06	100	284	3.24	5.54	69	85	
121-1	8	M			68 da.	30 da.	23.9	1236	0.35	0.63	76	1030	1.07	1.93	111	94	
2			48.5	56	3 mo.	2 mo.	20.8	340	2.62	4.82	105	243	4.42	8.13	127	116	
164	2.5	F	36		11 da.	5 da.	15.5	556	1.15	3.22	154	158	2.79	7.81	106	130	
59-1	9	M	52	53	29 da.	15 da.	25	509	2.16	3.50	95	625	1.33	2.15	72	84	
2			53	57	7½ mo.	7 mo.	30.2	360	3.96	6.26	100	633	1.93	3.05	85	93	
160	14	F	62	94	54 da.	39 da.	19.3	773	0.17	0.20	33	408	1.78	2.16	61	47	
5-1	12	M	57.5	95	4 mo.	3 mo.	21	256	1.65	2.31	38	512	1.23	1.72	59	49	
2			57.5	97	4½ "	3½ "	27	1557	0.82	1.15	115	410	4.73	6.62	134	125	
3			60.5	107	21 "	20 "	24.9	1345	1.11	1.41	119	375	3.93	4.99	100	110	

\* Since second acute attack.

† Time since acute symptoms.

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# KIDNEY FUNCTION DURING NORMAL PREGNANCY

## I. THE INCREASED UREA CLEARANCE OF NORMAL PREGNANCY<sup>1</sup>

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With the advent of urea and creatinine clearance tests as measures of kidney function, the possibility of more closely associating the etiology of the toxemias of pregnancy with renal pathology was reopened. Thus, Stander, Ashton and Cadden (1) undertook an investigation of renal function in the toxemias of pregnancy and determined the urea and creatinine clearance rates, in conjunction with a number of other tests of kidney function, on 65 women in various stages of pregnancy. They found no important variations in normal pregnancy or in the low reserve kidney of pregnancy. However, both clearances were subnormal, at from 55 to 80 per cent, in nephritic toxemias (Stander's classification (2)). Cadden and McLane (3) extended these studies on 343 pathological pregnancies, using urea and creatinine clearance and the phenolsulphonephthalein tests of kidney function, and concluded that only urea clearance was sufficiently sensitive to differentiate chronic nephritis from the other toxemias of pregnancy. Their antepartum averages were: for 9 cases of normal pregnancy, 122.7 per cent; for 90 of low reserve kidney, 101.7; for 87 of nephritis, 75.3; for 17 of preeclampsia, 84.4; and for 4 cases of eclampsia, 54.7 per cent. Hurwitz and Ohler (4), in 103 tests performed in the ninth month of pregnancy, found urea clearance values from 90 to 140 per cent, averaging 127 per cent, in normal pregnancy, and from 26 to 84 per cent in eclampsia, chronic nephritis and the late toxemias of pregnancy. There were a few high values in the last two groups. Cantarow

and Ricchiuti (5) determined 44 urea clearance rates on 39 cases of normal pregnancy. Seven were in the third, fourth and fifth months, 4 in the sixth, 5 in the seventh, 3 in the eighth, and 25 in the ninth month of pregnancy. Their clearance values varied from 28 to 184 per cent and appeared to fall from an average of 111 per cent in the third month to 59 per cent in the ninth month. In some recent work Dieckmann (6) found urea clearance decreased to averages below 50 per cent in patients with toxemia, hypertension or nephritis during the latter half of pregnancy. For normal pregnancy it averaged 102.3 per cent in 27 tests before delivery and 124.5 per cent in 10 tests immediately following delivery.

From these investigations it is apparent that the functioning level of the kidney during normal pregnancy must be more definitely established before its measurement during the toxemias can be of real value. A greater number of measurements should be repeated in series throughout normal pregnancies. The urea or the more difficult creatinine clearance test offers the most suitable method of accomplishing this end. Since Hayman, Halsted and Seyler (7) have found the results of the two tests completely comparable, the urea clearance appears to be the most practical test of kidney function available. Accordingly, the present work was undertaken to establish the changes of urea clearance rate through as long periods of normal pregnancy as possible.

### METHODS

A series of urea clearance tests, determined by the method of Möller, McIntosh and Van Slyke (8), was run on each of 13 normal pregnant women at intervals of two or three weeks. Several tests were obtained as early as the third and fourth months of gestation and as late as the eighth month postpartum. The tests were carried

<sup>1</sup> An abstract of this paper appears in the April, 1935, Proceedings of the American Society of Biological Chemists, J. Biol. Chem., 1935, 109, p. xlix.

<sup>2</sup> The data presented in this paper are taken in part from the thesis submitted by Margaret Nice to the Graduate School of Western Reserve University, June, 1934, in partial fulfillment of the requirements for the degree of Master of Science.



from 61 to 120 per cent, and the nonpregnant normals from 82 to 120 per cent. A statistical analysis, according to the procedures outlined by Dunn (12), shows that the variations of urea clearance from month to month during pregnancy are not significant; nor is the slight increase of 8.5 per cent from the second to the third trimester. The antepartum mean of 153 per cent, however, is significantly higher than the postpartum mean of 95.5 per cent or the nonpregnant mean of 105.0 per cent. The last two values are not significantly different and closely approximate Van Slyke's normal of 100 per cent. This establishes a definitely increased urea clearance rate during normal pregnancy, which is independent of the progress of pregnancy.

The standard deviation of 45 for the antepartum values is much larger than that of 18 for the postpartum results, or of 12 for the non-

pregnant normals. This wide variation occurs also in the rates for each individual.

The antepartum blood urea nitrogen values were low, averaging 7.7 mgm. per 100 cc., and showed a definite tendency to increase from an average of 6.2 mgm. in the third month to one of 8.3 mgm. in the ninth. The postpartum average was normal at 12.0 mgm. The relationship of the low antepartum blood urea to the high clearance is observed by plotting maximum clearance rates against their respective blood urea nitrogen values, as was done in Figure 1. The curve of the means of the clearances at successive blood urea levels indicates a rough inverse proportionality between the two.

When the blood urea nitrogen was raised by feeding urea, the same increased clearance rates were found in the second hour tests with the high blood urea nitrogen as in the first hour tests where it was low (Table II).

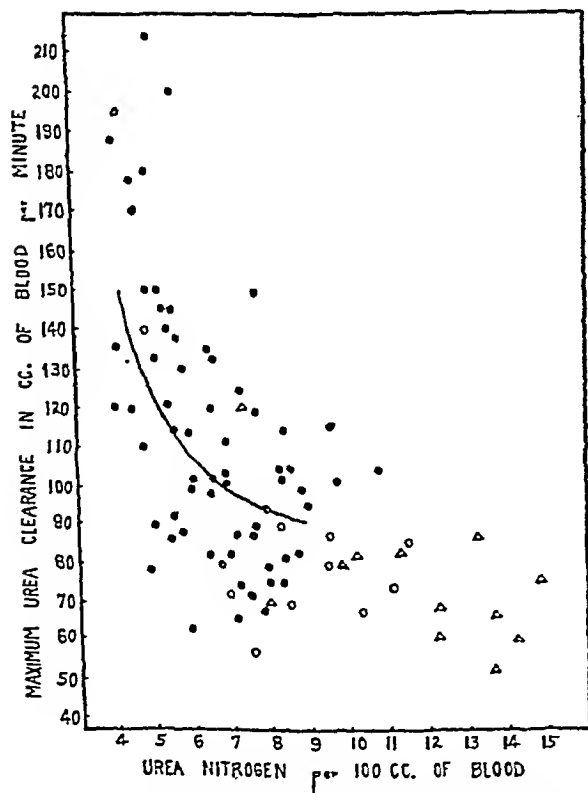


FIG. 1. THE RELATIONSHIP BETWEEN THE MAXIMUM UREA CLEARANCE RATES AND THE BLOOD UREA NITROGEN VALUES OF PREGNANCY.

● Antepartum      ○ Nonpregnant  
△ Postpartum      — Mean urea clearance

TABLE II

*Urea clearance rates before and after feeding urea*

Case number	Month of pregnancy	Blood urea nitrogen		Urea clearance rates	
		Before feeding urea*	After feeding urea* (Average of 2 determinations)	Before feeding urea*	After feeding urea*
		mgm. per 100 cc.	mgm. per 100 cc.	per cent	per cent
21	8	10.2	18.2	167	161
22	8	5.6	16.8	140	136
23	4	9.6	19.8	110	148
24	9	9.6	18.8	119	152
25	9	9.2	20.6	104	60
26	7	7.5	28.5	196	186
27	9	7.8	29.1	135	107
N-1	Nonpregnant	12.7	21.8	101	134
N-2	Nonpregnant	9.2	17.7	125	115

\* Patients 21 to 25, inclusive, and N-1 and N-2 received 10 grams of urea before the second test; 26 and 27 received 20 grams.

#### DISCUSSION

With the exception of the reports of Cantarow and Ricchiuti (5) and of Dieckmann (6), these results are in agreement with similar work of other investigators. The high urea clearance rate of pregnancy has been indicated by both Cadden and McLane (3) and by Hurwitz and Ohler (4).

The urea feeding experiments uphold the conclusions of Van Slyke et al. (13) and of Taylor, Drury and Addis (14), that urea clearance is unaffected by the blood urea level.

The low antepartum blood urea nitrogen values have been found previously by other workers (4, 15) and could be considered as the result of an increased excretion of urea. This interpretation is emphasized by the concurrence of the high antepartum urea clearance values with the low blood urea.

The standard deviation of 45 for the urea clearance rates of pregnancy appears to be an exaggeration of the normal nonpregnant clearance variation, which was first pointed out by Bruger and Mosenthal (16). This exaggeration parallels the increase in the clearance rate for pregnancy.

Conclusions cannot be adequately drawn from the present work until the daily variation of kidney function during pregnancy has also been established. Work along these lines is now in progress, and further discussion is reserved until a later paper.

#### SUMMARY

The urea clearance test was run 93 times on 13 normal pregnant women from the fifth month of pregnancy to the eighth month postpartum; 10 times on 4 normal nonpregnant women; and also before and after feeding urea to 7 pregnant women.

The mean antepartum urea clearance found is 153 per cent of normal. This is significantly higher than the postpartum mean of 95.5 per cent, or than the mean of 105 per cent for the nonpregnant normals. The standard deviations for the three groups are 45, 18 and 12, respectively.

The rough proportionality between the low blood urea and the high urea clearance of pregnancy is of considerable interest and suggests the dependence of the former upon the latter.

Raising the low blood urea by feeding urea did not alter the high urea clearance.

I am indebted to Dr. Edward Muntwyler for advice and criticism throughout this work.

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# SERUM LIPOIDS IN DIABETES

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Recently high-carbohydrate, low-fat diets have been widely advocated for diabetic patients (3, 17, 20, 23, 24, 30, 36, 48, 52). As arguments for this therapy it has been claimed that hypercholesterolemia is of grave prognosis in diabetes and that high fat diets may superinduce hyperlipemia. The following investigation was undertaken to determine the incidence of hypercholesterolemia in diabetes, to study the relation of the cholesterol level to that of fatty acids and phospholipoids in blood serum, and also to learn whether diets moderately high in fat such as are used in this clinic give rise to hyperlipemia.

## MATERIALS AND METHODS

The serum, from venous blood taken before the morning insulin or breakfast, of 79 different diabetic patients, 20 males and 59 females of all ages, has been examined 130 times for cholesterol, lipid phosphorus and non-phospholipoid fatty acids by methods already described (37, 38). The percentage errors of the analytical techniques have been discussed previously (37, 38, 40). In almost every instance serum proteins (11) and blood sugar (4, 54) were determined simultaneously. No studies which were made when the patients were dehydrated, acidotic, pregnant, postpartum, or had been treated with intravenous injections of acacia are included.

## DATA

Data are given in Table I. Insulin dosage and protein, fat and carbohydrate intake indicate the requirements and food consumption of the patient for at least four or five days previous to the blood study. Whenever there was any doubt that the diet had been followed adequately, the term "unregulated" has been used. The blood of many of these patients was examined the morning after admission to the hospital, and unless there was an exact record of the diet the

regimen was condemned to the "unregulated" category. Brief protocols concerning each patient are presented at the end of the paper. Notes are included where there were evidences of hypertension or arteriosclerosis, the latter being judged by evidences of sclerosis in the eyegrounds and extremities, cardiac enlargement and decompensation. Blood pressures were observed on all patients, not always on the day of the blood study, but when the subject was neither unduly excited nor in circulatory embarrassment. In addition to the actual body weight, the impression of nutritional state is described. Any evidences of liver pathology or enlargement, or of kidney pathology, are included, but neither organ is mentioned unless an abnormality was discovered. The albumin and globulin in the serum of more than half the patients were determined. In most instances any alteration in total protein was due to a variation in albumin, but in certain cases when the albumin:globulin ratio was abnormal the concentrations of the fractions are included.

Cases have been grouped according to the concentration of cholesterol in the serum. Whenever this fluctuated the study used for classification was selected when the condition of the patient was most nearly normal or when the cholesterolemia had attained a constant level. The table includes first the patients who exhibited hypercholesterolemia, secondly those with hypocholesterolemia and lastly those with normal cholesterolemia. The first subdivision of the hypercholesterolemia group includes subjects with greatly elevated cholesterols or with flagrant complications; and the second group, those relatively uncomplicated diabetics whose serum cholesterols were between 250 and 304 milligrams per cent. An attempt has been made to arrange the subjects so that those with similar complications are listed together. In the group with normal cholesterolemia the first thirteen patients had no evident complications.



TABLE I

Case number	Date	Serum					Blood sugar	In-sulin	Diet		
		Cholesterol	Lipoid phosphorus	Non-phospho-lipoid fatty acids	Total fatty acids	Protein			Protein	Fat	Car-bohy-drate
		mgm. per 100 cc.	mgm. per 100 cc.	m. eq.	m. eq.	per cent	mgm. per 100 cc.	units	grams	grams	grams
A. HYPERCHOLESTEROLEMIA											
1. Severe hypercholesterolemia and patients with severe complications											
A8513	November 11, 1932	421	18.2	48.9	60.7	6.23	240	115	70	125	125
	November 19, 1932	335	17.7	62.0	73.4	6.78	216	150	70	125	125
	November 25, 1932	459	21.7	80.1	94.1	6.96	189	205	90	150	125
	November 26, 1932	389	19.5	65.2	77.8		176	220	90	150	125
	December 6, 1932	410	18.3	48.2	60.0		182	270	90	150	125
	December 12, 1932	395	21.1	67.1	80.7	6.97	183	160*	90	150	125
	December 20, 1932	326	16.0	56.2	66.6	6.50	180	245	90	150	125
A10517	March 31, 1933	290	12.9	17.7	26.0	7.13	138		—Unregulated—		
	April 7, 1933	271	11.3	13.5	20.8		150	30	68	85	86
	January 10, 1934	225	10.7	12.9	19.8	7.26	163	30	—Unregulated—		
5496	March 21, 1933	238	11.1	12.9	20.0	5.38		15	25	43	51
	April 12, 1933	289	12.1	14.2	22.0	5.25		15	29	76	72
A1231	January 10, 1934	358	14.3	13.4	22.6	4.83	167	20	50	108	105
	January 23, 1934	258	11.3	12.3	19.5	4.27	243	15	48	91	88
	February 28, 1934	321	14.0	16.4	25.4	3.93		20	59	126	81
	March 5, 1934	414	15.6	18.6	28.7	4.53		15	81	160	124
	March 26, 1934	520	17.1	18.7	29.7	3.61		40	78	137	117
	April 18, 1934	620	20.7	28.2	41.5	4.74	254	30	66	96	101
	May 10, 1934	684	24.7	37.6	53.5	4.51		15	83	161	147
	May 31, 1934	456	19.8	27.6	40.4	4.57		50	106	147	137
	June 21, 1934	423	14.6	22.2	31.6	4.98		50	84	119	127
17383	November 2, 1933	280	12.0	14.5	22.2	7.67	252	0	—Unregulated—		
	November 7, 1933	307	11.8	14.9	22.5	7.71	271	0	50	125	125
	November 16, 1933	269	11.8	14.8	22.4	7.75	270	0	57	132	135
70975	February 26, 1934	355	13.9	10.1	19.1	6.57		0	—Unregulated—		
A31776	October 28, 1933	288	12.7	16.0	24.1	6.31	331	135	70	150	125
A9255	January 5, 1933	194	10.4	19.7	26.4	6.33	341	240	70	125	150
	March 16, 1933	515	18.5	38.3	50.2	6.53	362	200	90	175	150
	March 27, 1933	514	21.7	35.1	49.1			200	90	175	150
	April 27, 1933	444	22.3	35.2	49.6		345	215	90	175	140
	June 15, 1933	355	14.3	18.6	27.9	6.70	355	230	90	175	140
	July 21, 1933	384	15.8	22.6	32.7	6.34	417	170	90	175	140
	March 23, 1934	366	16.5	19.5	30.2	6.96	387	125	80	175	140
	February 5, 1935	382	20.4	36.6	49.8	6.87	138				
A8380	October 29, 1932	281	14.3	14.2	23.5			60	Vomiting		
	November 17, 1932	386	17.2	20.5	31.5			60	70	175	150
A30304	May 4, 1933	615	21.1	15.4	29.0	5.81	293	5	Unmeasured		
	May 10, 1933	465	15.8	12.4	22.6	5.59	266	100	70	125	100
	May 19, 1933	371	17.9	11.3	22.9	5.90	339				
A52638	January 5, 1935	239	11.6	10.9	18.5	7.17					
	January 25, 1935	270	14.1	11.1	20.2	6.64		25	90	200	250
A53920	April 12, 1935	312	13.1	13.5	22.0	6.90		0	50	60	60
69743	April 16, 1935	340	15.4	11.1	21.0	6.65		60	90	200	150
A32114	November 1, 1933	513	16.4	18.7	29.2	5.68	277	0	Starvation		
	November 4, 1933	374	14.2	12.0	21.2	5.22		90	80	175	150
	November 10, 1933	258	10.8	9.3	16.2	5.32	282	85	80	175	150
	November 17, 1933	260	11.5	8.9	16.3	5.43	273	70	80	175	150
34878	August 30, 1933†	296	14.3			5.88	137	60	70	125	125
1898	March 30, 1933	306	11.3	11.3	18.6	6.52	176	25	70	125	125
A9112	January 16, 1933	213	11.6	8.4	15.9	7.29	240	115	70	150	125
	January 26, 1933	272	13.1	10.5	18.9			60	100	150	125
	February 1, 1933	270	13.5	8.8	17.6		232	50	100	150	125
A7358	September 23, 1933	276	12.1	11.9	19.7		355	125	80	150	175
	September 28, 1933	304	13.1	11.3	19.8	7.07	325	130	80	150	175
36671	May 20, 1933	391	15.1	16.9	26.7	6.58	294	20	Unregulated		

\* Beef insulin.

† 3 P.M.

TABLE I—Continued

Case number	Date	Serum					Blood sugar	In-sulin	Diet		
		Cholesterol	Lipoid phosphorus	Non-phospholipoid fatty acids	Total fatty acids	Protein			Protein	Fat	Carbohydrate
		mgm. per 100 cc.	mgm. per 100 cc.	m. eq.	m. eq.	per cent	mgm. per 100 cc.	units	grams	grams	grams
2. Mild hypercholesterolemia											
2854	February 21, 1933	245	10.4	14.9	21.6		205		—Unregulated—		
	February 24, 1933	245	10.3	12.8	19.4		212	75	68	140	143
	March 9, 1933	273	10.7	13.4	20.3	6.76	163	30	70	150	150
33222	May 17, 1933	270	12.1	8.1	15.9	7.47	233	40	—Unregulated—		
33580	June 27, 1933	274	10.9	11.7	18.7	6.19	196	10	—Unregulated—		
A9452	February 8, 1933	270	12.3	10.7	18.6		209	10	70	125	125
A14006	February 7, 1933	265	10.4	14.0	20.7		149	45	—Unregulated—		
A30212	April 27, 1933	304	12.2	9.4	17.3	7.95	121	25	68	140	93
A31035	July 14, 1933	244	11.0	16.8	23.9	7.00	179		—Unregulated—		
	July 21, 1933	261	10.1	9.0	15.5	6.76	285	25	70	125	125
A32422	December 2, 1933	277	12.7	11.7	19.9	6.93	222	0	—Unregulated—		
A53097	March 9, 1934	287	11.0	7.9	15.0	6.60	160	0	70	150	125
B. HYPOCHOLESTEROLEMIA											
29923	March 21, 1934	117	6.2	6.4	10.4	5.17			—Unregulated—		
33395	March 14, 1934	85	6.0	3.0	6.8	4.69	110		—Unregulated—		
46000	April 2, 1934	51	3.8	3.2	5.6	4.72	51		—Unregulated—		
	April 23, 1934	92	5.7	5.0	8.7	5.99		10	30	44	118
A8983	December 29, 1932	119	9.9	8.6	15.0	6.05	217		—Unregulated—		
	December 31, 1932	150	11.2	7.5	14.8	6.36	127	60	79	146	118
	January 6, 1933	135	8.8	7.5	13.2	5.60	150	50	81	125	174
	February 14, 1933	149	7.5	7.7	12.4	7.12	128		110	125	220
A9017	December 31, 1932	139	7.1	6.6	11.2	6.37	312		—Unregulated—		
A30909	June 29, 1933	117	5.6	4.5	8.1	6.39	97		—Unregulated—		
A32215	November 15, 1934	90	7.3	9.7	14.4	4.87	207		Carbohydrate restricted (unmeasured)		
	December 29, 1934	122	8.2	11.3	16.6	5.58	108	20	45	99	91
A32655	December 27, 1933	105	6.0	5.2	9.1	5.09	151		—Unregulated—		
A32829	January 14, 1934	83	5.8	6.8	10.5	5.01		15	Only fruit juice and beef broth		
	January 15, 1934	80	5.1	5.6	8.8	4.70	362				
C. NORMAL CHOLESTEROLEMIA											
1. Uncomplicated diabetics											
29176	March 21, 1933	216	11.4	16.8	24.2	6.40	375		—Unregulated—		
	May 13, 1933	194	9.8	10.4	16.8	5.95	446	100	70	125	125
	May 16, 1933	197	10.4	10.9	17.6	5.87	446	80	70	125	125
36052	September 19, 1933	224	10.2	13.0	19.6	7.37		0	—Unregulated—		
43494	July 7, 1933	248	9.5	9.5	15.7	7.12	231	10	—Unregulated—		
45815	October 28, 1932	209	11.4	10.5	17.9	6.56	275	85	70	150	125
60404	January 3, 1933	155	7.5	10.3	15.2	5.63	425	60	90	151	150
A6028	October 11, 1932	212	9.4	6.4	12.5			14	90	200	125
A14670	July 8, 1933	195	7.3	6.5	11.1	6.65	230		—Unregulated—		
A26960	June 23, 1933	168	7.5	8.4	13.2	7.22	186		—Unregulated—		
A31733	September 23, 1933	220	9.5	9.9	16.1	7.04		0	Restricted carbohydrate		
A32622	January 4, 1934	253	11.4	11.2	18.5	6.43	196	35	60	100	125
A33706	April 25, 1934	236	11.2	8.4	15.6	5.98	198	40	70	170	125
A36853	February 24, 1934	207	10.4	6.5	13.2	6.98	254	25	90	200	200
A42040	May 29, 1934	188	8.4	7.0	12.4	6.25	218	55	71	120	99
									Unregulated until May 25		

TABLE I—Continued

Case number	Date	Serum					Blood sugar	In-sulin	Diet		
		Cholesterol	Lipoid phosphorus	Non-phospho-lipoid fatty acids	Total fatty acids	Protein			Protein	Fat	Car-bohy-drate
		mgm. per 100 cc.	mgm. per 100 cc.	m. eq.	m. eq.	per cent	mgm. per 100 cc.	units	grams	grams	grams
2. Complicated diabetics											
963	April 29, 1933	247	12.7	9.6	17.8	7.25	278	0	—Unregulated—		
5105	April 12, 1933	200	8.1	7.6	12.8	6.50		35	80	135	121
18576	February 8, 1933	249	10.8	10.4	17.3			0	46	41	38
29503	January 16, 1934	263	11.1	9.0	16.1	5.86	240	40	80	150	130
	February 19, 1934	165	8.3	6.7	12.1	6.04	224	10	21	50	70
	March 13, 1934	248	11.4	10.4	17.7	5.72	288	10	0	0	0
34442	June 27, 1933	206	9.9			7.16	285		—Unregulated—		
36572	December 18, 1933	152	7.8	5.2	10.3				—Unregulated—		
40992	June 28, 1933	183	8.1	7.4	12.6	6.30		10	—Unregulated—		
15096	February 6, 1934	250	10.7	8.2	15.1	6.51		27	—Unregulated—		
58487	February 18, 1933	210	10.0	10.6	17.1		220	45	—Unregulated—		
61682	January 16, 1933	170	9.2	6.1	12.1	6.74	232	30	91	200	126
79627	February 3, 1934	155	8.8	7.7	13.3	6.94	129	0	44	39	79
83790	November 2, 1932	169	8.9	9.4	15.2		243	0	—Unregulated—		
83896	March 1, 1933	232	14.9	16.7	26.3		80		—Unregulated—		
	March 3, 1933	226	11.8	13.5	21.1	6.23	259	55	60	99	99
	March 14, 1933	255	10.2	11.5	18.1	7.96	195	65	60	100	101
85804	June 2, 1933	208	8.2	7.1	12.4	5.97		0	Carbohydrate - restricted		
90339	November 4, 1932	142	8.0	9.6	14.8	5.50	302	40	70	150	125
94028	February 22, 1934	175	9.1	7.9	13.7	7.86	185	0	70	100	125
96675	December 28, 1933	234	10.0	10.3	16.7	6.15	123	0	70	150	100
										(Diet poorly regulated)	
A7948	November 3, 1933	161	8.1†	9.5†	14.7	6.01	138		—Unregulated—		
A8517	November 14, 1932	226	10.7	10.9	17.8			25	64	111	86
A9315	February 21, 1933	252	9.6	9.0	15.2				—Unregulated—		
A15928	April 4, 1933	208	9.5	8.4	14.5	6.67		15	75	125	50
A29051	September 8, 1933	189	9.0	9.6	15.4	6.95	246	45	—Unregulated—		
A30054	April 12, 1933	210	9.8	9.2	15.0			0	67	99	110
A30790	June 20, 1933	148	7.5	11.6	16.4	7.17	229	60	—Unregulated—		
A30940	July 19, 1933	154	8.8	7.2	12.9	6.03	381	80	79	176	125
	September 12, 1933	204	8.5	6.8	12.2	6.51	273	80	100	250	165
	September 29, 1933	220	10.5	7.6	14.4	7.29	321	85	100	225	165
A32367	December 5, 1933	201	8.7	9.9	15.5	6.70		50	27	75	77
A32692	April 10, 1934	223	9.7	11.1	17.4	6.94		15	53	120	133
A33062	February 9, 1934	195	8.7	7.9	13.5	7.08	290		—Unregulated—		
A34635	July 14, 1934	191	8.0	6.7	11.7	5.80	120	0	80	175	175

† One determination.

In Figures 1 and 2, cholesterol is compared with non-phospholipoid fatty acids and with lipoid phosphorus respectively. Only one determination from each of the seventy-nine patients has been included. Whenever a long series of studies has been made the final determination has been selected because most patients were best regulated at the time of the last study. The range of normal values is indicated on each chart by an enclosed area.

## OBSERVATIONS

*Infrequency of hypercholesterolemia in diabetes*

Of the 79 patients, serum cholesterol was normal in 42, above normal in 28, and below normal in 9. The normal range for serum cholesterol is considered to be 150 to 256 milligrams per cent (38), although at present an attempt is being made to ascertain whether the range for females is the same as that for males. Such an analysis

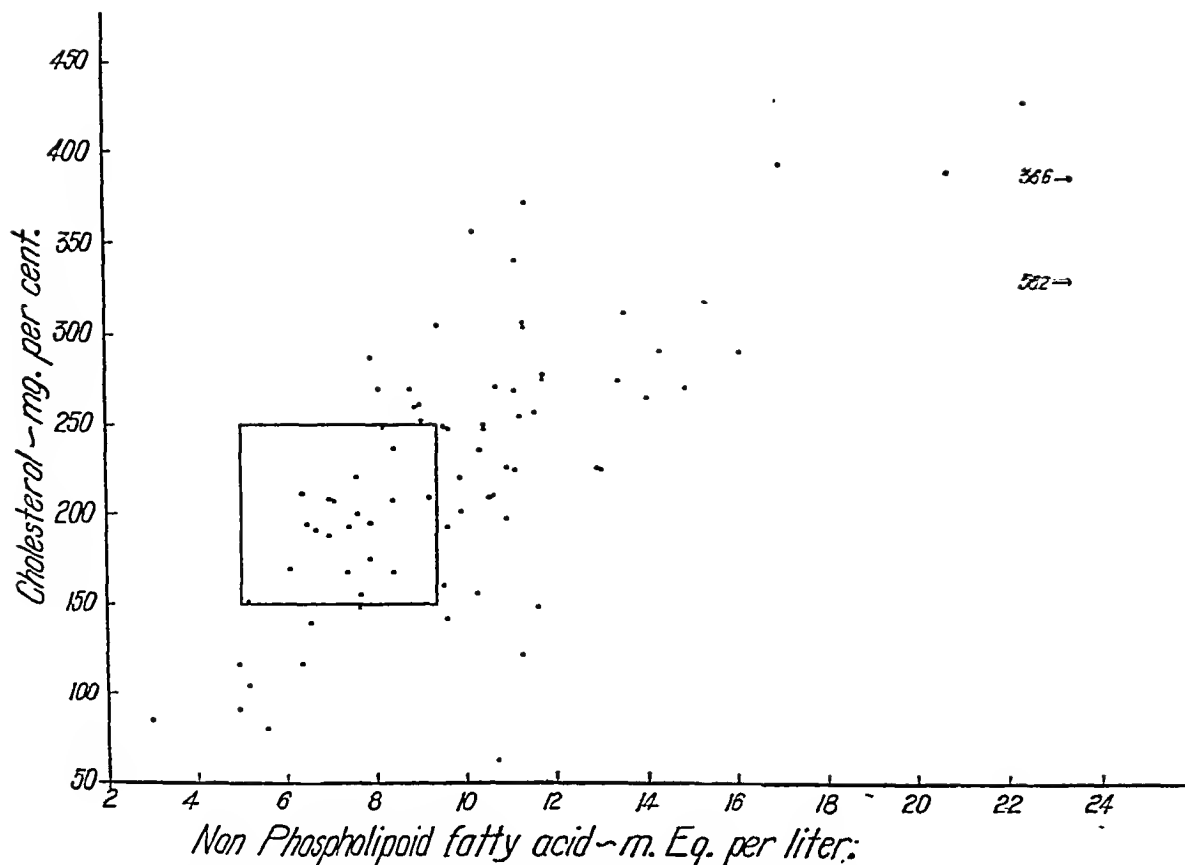


FIG. 1. RELATION BETWEEN CHOLESTEROL AND NON-PHOSPHOLIPOID FATTY ACIDS OF SERUM.

Only the last determination from each subject is given. The square defines the limits of normal variation.

shows that hypercholesterolemia in diabetes is less frequent than is generally supposed. Of the 28 patients with hypercholesterolemia all were females, except two (A8513, A10517) with liver cirrhosis, one (70975) with nephrosis, and one (A53920) with vasomotor instability, even though about 27 per cent of the patients studied were men.

Of the first nineteen patients who had hypercholesterolemia of considerable severity, all but three had complications which may have been largely or entirely responsible for the lipid disturbance. Two (A8512 and A10517) were suffering from cirrhosis, which is notoriously associated with disorders of cholesterol metabolism (18, 55). Four (5496, 17383, 70975, A1231) had severe kidney disease, the two last with frankly nephrotic aspects. A31776, at the time of the recorded observations, had a generalized xanthomatous eruption. The next seven cases

make up a rather peculiar group. The first, A9255, had acromegalic diabetes with extreme symptoms of autonomic instability, suggesting involvement of the hypothalamus: nervousness, flushing, excessive sweating and tachycardia. In addition, her basal metabolism was greatly increased and remained elevated after subtotal thyroidectomy. A32114, A30304, A8380, A52638, A53920 and 69743 exhibited similar symptoms and signs of sympathetic irritability without definite stigmata of pituitary disease. The basal metabolisms of the last five were also increased, and that of A52638 failed to respond adequately to thyroidectomy, until five weeks after the last determination of blood lipoids which had been made ten days after the second operation of a two-stage thyroidectomy. A32114 had subsisted for some time on an extremely limited diet. Since the hypercholesterolemia subsided within nine days under treatment, it may have been connected with

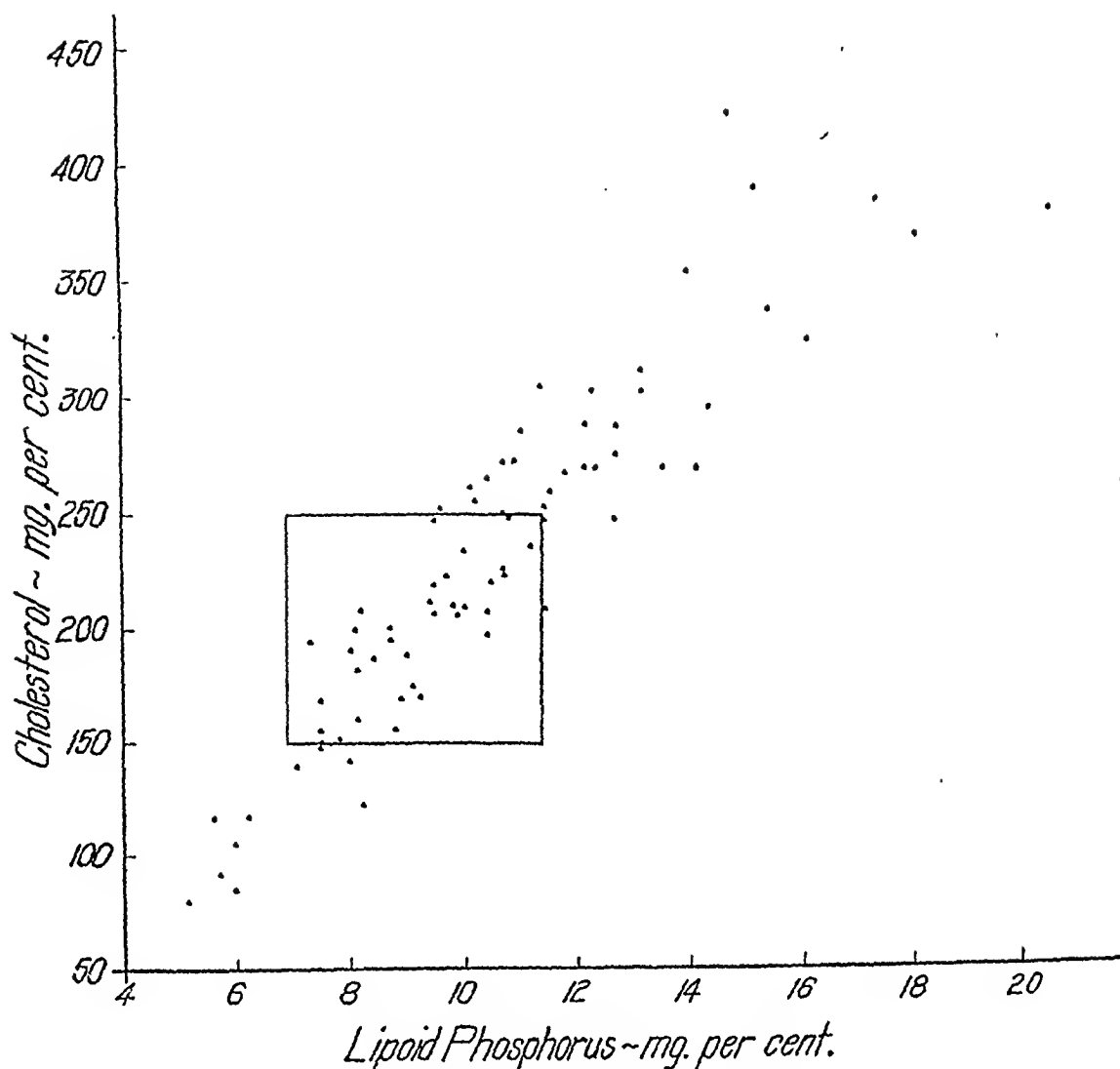


FIG. 2. RELATION BETWEEN CHOLESTEROL AND LIPOID PHOSPHORUS.

Only the last determination from each subject is given. The square defines the limits of normal variation.

some acute disturbance of undiscovered nature. The patient had shown large amounts of acetone in the urine (2+ to 4+) for the two days after admission to the hospital and prior to the initial blood study. Of the next two patients, 34878 had Parkinson's disease, while 1898 had diabetes insipidus.

For the hypercholesterolemia in the three remaining cases of the first group no distinct complications can be held responsible. A9112 had pernicious anemia and vague symptoms of vasomotor instability, such as slight tachycardia and unstable carbohydrate metabolism. A7358, a child of 13, was studied after recovery from severe acidosis, which had been precipitated by an acute exacerbation of a chronic purulent otitis media.

Most of the nine patients, all females, in the next group with moderate hypercholesterolemia presented minor complications: cataracts, retinitis, cholelithiasis, infections, etc. 2854 might easily be included in the normal group, but as her cholesterol rose with improvement from 245 to 273 milligrams per cent, the latter was selected as the fairest criterion of her "normal" cholesterolemia. All the patients in this group were frankly overweight or somewhat overnourished.

#### *Hypocholesterolemia*

A8983, one of the 9 patients with hypocholesterolemia, had hypertrophic cirrhosis of the liver and should be omitted from the series because cirrhosis may, according to Epstein (18) and Thannhauser and Schaber (55), cause either

elevation or diminution of cholesterolemia. This leaves eight patients with striking hypocholesterolemia. All were markedly emaciated or malnourished, often with low serum albumin and massive edema, like that of A32215 who had been on a fluid diet for six days following an appendectomy complicated by peritonitis.

#### *Cholesterolemia as a criterion of lipemia*

In Figure 1 cholesterol have been charted against non-phospholipoid fatty acids. Immediately it is apparent that the fatty acids may be distinctly above the normal maximum, even when the cholesterol is not. The number of cases in which this occurs is much greater than the number of cases in which a hypernormal cholesterol is associated with fatty acids within the normal range. Also the fatty acids may be within the normal range when the cholesterolemia is below normal, although normal cholesterol has not been found with subnormal fatty acids. The independence of variations in cholesterol and fatty acids has already been discussed in a previous article on diabetic acidosis (40) and is in contrast to the findings of certain other investigators (7, 9, 15, 27, 46, 47, 59).

That the concentrations of cholesterol and lipid phosphorus in blood serum bear a distinct relation to each other is clearly demonstrated in Figure 2. This has already been suggested by Bloor (8). A graphic comparison of cholesterol and total fatty acids shows only lack of correlation, like that between cholesterol and non-phospholipoid fatty acids. This might easily be deduced from a study of Figures 1 and 2. Similarly it may be argued from these same figures that lipid phosphorus and non-phospholipoid fatty acids may vary independently.

#### DISCUSSION

The statistical correlation between lipid phosphorus and cholesterol in blood serum is quite close. Although the correlation between serum cholesterol and fatty acids is far less regular, there is a general tendency to correlation, which is evidenced in the fact that no markedly elevated fatty acids were found in the groups with cholesterol normal or below normal, and that fatty acids were consistently above normal in those with marked hypercholesterolemia. For these reasons

generalizations in this discussion in regard to cholesterolemia and phospholipoidemia are applicable also to lipemia. No attention has been given to ratios of lipid constituents because of the uncertainty concerning the functional relationship of the various fractions. It is not surprising in light of the differences in constitution and properties of fatty acids and cholesterol, that in spite of the general correlation between these two substances independent variations are observed. Such dissimilar substances must be metabolized by different processes. For example, a marked increase in serum fatty acids after the ingestion of food is not accompanied by a rise in serum cholesterol (2, 5, 6, 7, 13, 14, 19, 26, 33, 41). Similarly, the increase in fatty acids in diabetic acidosis far exceeds the elevation of cholesterol (40). In Figure 1, in which cholesterol and non-phospholipoid fatty acids are compared, the lack of correlation is partially masked because the non-phospholipoid fatty acids have not been corrected for the fraction combined with cholesterol. In the cholesterol esters about seventy per cent of the cholesterol (10, 18, 35, 45, 55) and about half the non-phospholipoid fatty acids are chemically combined.

Our data show that hypercholesterolemia of uncomplicated diabetics did not exceed 304 milligrams per cent, and also that cholesterol between 250 and 304 milligrams per cent were only encountered in 9 of 79 diabetics. The explanation for the low incidence of hypercholesterolemia undoubtedly depends on the fact that there have been omitted from this series all diabetics with dehydration or acidosis. The justification for such omissions rests upon a previous investigation, in which it was demonstrated that dehydration was largely responsible for the hypercholesterolemia of diabetic acidosis (40). It has been shown that the capillaries are impermeable to both serum proteins and cholesterol (39) and that hemoconcentration causes the concentration of both these constituents to increase. For example, the alleviation of dehydration of one acidotic patient (90339), when the serum proteins fell from 7.92 to 4.87 per cent, would account for a diminution of serum cholesterol from 275 to 169 milligrams per cent (40). The relation of lipoids to proteins of serum, and consequently to blood volume, in patients without diabetic acidosis has already been considered (39, 40). In this series, no pa-

tient has been included who showed clinical symptoms of dehydration or who immediately after hospitalization had a limited urine output in relation to fluid intake. Such dehydrated patients may have been included in earlier studies of diabetic cholesterolemia. Insufficient attention may also have been given to the complications which so commonly accompany diabetes.

Especial virtue has been ascribed to the determination of serum cholesterol as a measure of the severity of diabetes. When patients with obvious acidosis are excluded, as they are in this series, there is no discoverable relation between serum cholesterol and the gravity of the disease, measured either in terms of insulin requirement or carbohydrate tolerance. Most of the patients with hypocholesterolemia were extremely ill and profoundly emaciated. As a group the patients with mild hypercholesterolemia did not require excessive amounts of insulin: the maximum requirement after regulation was 45 units daily, one (A53097) required no insulin, two (33580, A9452) required only 10 units of insulin daily, and two (A30212, A31035) 25 units; while in the group with normal cholesterols were 29176 taking 100 units of insulin daily, 45815 using 85 units of insulin, A30940 using 80 units, 83896 using 65 units and A42040 on 55 units.

It is quite as impossible to relate either cholesterolemia or lipemia to fat intake. Within the normal range were the fatty acids and cholesterol of 61682, A6028, A30940 and A36853, all of whom ate 200 grams of fat daily, of A33706 and A34635 who had 170 and 175 grams of fat respectively, and of 45815 and 90339 who had 150 grams of fat; while of the 9 patients with mild hypercholesterolemia, 2 had 150 grams of fat daily; 1, 140 grams; 2, 125 grams; and 4, unregulated amounts of fat. A32114, who had been on a starvation diet, has been mentioned previously. During ten days of a diet consisting of 80 grams of protein, 175 grams of fat and 150 grams of carbohydrate, her cholesterol fell from 513 to 259 milligrams per cent and her total fatty acids from 29.2 to 16.2 milli-equivalents. One week later, after continuance on the same diet, her lipoids were at precisely the same level. A30940, who entered the hospital in July, 1933, in a profoundly malnourished condition, went through a

stormy course of pleurisy with effusion and staphylococcus abscesses of the areolar tissue. On July 12, she was placed on a diet containing 175 grams of fat and 2400 calories, which was later increased to a diet containing 200 grams of fat, 90 of protein and 150 of carbohydrate. About September 12 this was changed to 250 grams of fat, 100 of protein and 165 of carbohydrate, and was reduced to 225 grams of fat before September 29. As the malnutrition was alleviated, in spite of this high fat diet, the cholesterol rose only to 204 and 200 milligrams per cent on September 12 and 29, and the fatty acids only to 12.2 and 14.3 milli-equivalents on these dates.

Among cases with rapidly changing diets the discrepancy between cholesterolemia and lipemia is most marked but is not related to the fat in the diet. Hyperlipemia of a mild degree occurred simultaneously with normal cholesterols in certain patients, 963, 18576, 29176, 29503, 36052, 58487, 83896, who had been on an unregulated diet or who had abandoned a diabetic regimen. A31035 is an outstanding example of this, for before the initial study on July 14, 1933, diabetes had not been diagnosed and no limitations of diet had been prescribed. At this time her fatty acids were 23.9 milli-equivalents although they fell to 15.5 milli-equivalents after seven days on 125 grams of fat and 1900 calories. During this interval the cholesterol did not follow the fatty acids but increased from 244 to 261 milligrams per cent.

The argument which has been advanced in behalf of high-carbohydrate, low-fat diets, that high-fat diets tend to produce hypercholesterolemia, has not been substantiated by other investigators. Curtis, Sheldon and Eckstein (16) in a case of diabetes with xanthomatosis and lipemia retinalis noted a reduction in total blood lipoids from 1.780 per cent to 1.400 per cent on a diet of 55 grams of protein, 300 grams of fat and 270 grams of carbohydrate when sufficient insulin was administered to insure aglycosuria and normal blood sugar. Rabinowitch (49) reported 500 cases, the second 250 of which were treated with his new low-fat, high-carbohydrate diet. His data show that only 76 on the new diet, in contrast to 118 on the older diet, had cholesterols ranging between 151 and 200 milligrams per cent and that only 31 of Group II, instead of 40 of Group I, had cho-

lesterols between 200 and 250 milligrams per cent. Whether this reduction in cholesterolemia is important is questionable because many investigators have found that cholesterols of normal subjects vary within the limit of 150 to 250 milligrams per cent. Nevertheless, on this new high-carbohydrate, low-fat diet, 14 patients with cholesterolemia of 250 to 200 milligrams per cent were observed, while only 5 were noted in the group ingesting the older diet. Similarly 11 of Group II, in contrast to 6 of Group I, had serum cholesterols above 301 milligrams per cent. Bruger and Poindexter (12) have found that high-fat, high-calorie diets, productive of obesity, are not associated with increases in plasma cholesterol.

The tendency to obesity of the nine subjects with slightly elevated cholesterols must be considered as peculiar to the individual rather than as the result of a large fat intake. It seems probable that these cholesterols between 250 and 304 milligrams per cent are more closely related to the sex and body build (22, 51, 59) than to the diabetic condition. Bruger and Poindexter, while studying cholesterolemia in relation to 94 patients who were more than twenty per cent overweight, had in their series only 4 males, but 90 females (12). This seems to indicate that an unusual lipid metabolism, one manifestation of which is an elevation in serum cholesterol, may be associated with a particular form of obesity often observed in females.

Cachexia has long been thought to be accompanied by a low level of cholesterolemia. The finding that all of the patients with hypocholesterolemia were extremely emaciated and had low serum albumin indicates a distinct relation between nutrition and the level of serum cholesterol. To demonstrate the converse, that all emaciated patients have a low cholesterol, is more difficult because the degree of emaciation is hard to define and it is difficult to differentiate a person of lean build who has lost a little weight from a subject with more pronounced tissue wasting. For example, A33706, with a serum cholesterol of 236, was extremely thin but showed no muscular wasting. In spite of her undernourished appearance the serum albumin was 4.50 per cent, although it has already been shown that severe emaciation is attended by a diminution in serum albumin (44).

Again, 61682 was extremely thin but was of a spare physique and had a serum cholesterol of 170 milligrams per cent and an albumin of 4.14 per cent, both within the normal range. In the hypercholesterolemia group there are two patients (A30304, A32114) with profound emaciation who had cholesterols above normal when the wasting was most pronounced. Some unrecognized factor may here affect the cholesterolemia, just as some unrecognized factors produce hypercholesterolemia in nephrosis even when there is profound malnutrition.

On account of conflicting observations (1, 21, 31, 32, 34, 43, 50, 53) in regard to cholesterolemia and arteriosclerosis, the clinical picture of every diabetic in Figure 1 was scrutinized to evaluate the degree of vascular degeneration. Lipoid values and blood pressure were also compared. It was found that severe arteriosclerosis, with or without hypertension, was evident in patients with serum cholesterols normal, below normal and above normal.

In all problems connected with diabetes a distinction must be drawn between those phenomena which are associated only with the disturbance of carbohydrate metabolism and those which are characteristic of the underlying disease. This is the more necessary since it has become evident that the pancreas alone need not be held responsible for all cases of diabetes. From this point of view the chief impression derived from this study is that abnormalities of serum lipoids are related loosely, if at all, to diabetes. Certainly hyperlipemia and hypercholesterolemia are not a regular part of the diabetic syndrome and, when they do occur, cannot be connected with the inherent severity of the disease, measured by any recognized criteria. In diabetic acidosis a relative or absolute hyperlipemia is regularly observed, which diminishes or disappears with the acidosis. It was pointed out in an earlier communication (40) that this hyperlipemia is largely, but not entirely, a product of hemoconcentration. During recovery, the reductions of the various lipid fractions were not exactly proportional to one another nor to the fall of serum proteins. This suggested that these fractions were influenced in part, and to different degrees, by something other than hemoconcentration. In general, the relative magnitudes of



the reductions took the following order: non-phospholipid fatty acids > phospholipids > cholesterol > protein. This is the order of importance conventionally ascribed to these components in fat metabolism. The increases in excess of the effects of hemoconcentration may, then, represent merely the mobilization of fat to meet an emergency demand in a condition in which utilization of carbohydrate is reduced to a minimum, if not abolished.

The patients considered in this paper were studied in conditions in which, although carbohydrate tolerance was impaired, they were utilizing sugar. To be sure the actual proportion of carbohydrate in the metabolic mixtures was less than that in the ordinary dietary of normal persons. But this would seem to have had little influence upon lipemia, since this cannot be correlated with diet in any respect. The amount of carbohydrate presumably burned was no greater among those with hypolipemia than among the hyperlipemic. In treated diabetics, then, it would seem necessary to seek the causes of disturbances of lipemia in peculiar features of the individual patterns of the underlying disease. In a certain proportion, adequate explanations for hyperlipemia can be found in complications or associated diseases such as cirrhosis of the liver, nephrosis or other renal disorders.

When these are eliminated it is found that most of the remainder with greatly elevated lipoids fall into a group that presents one common feature, which may be roughly spoken of as instability of the autonomic nervous system. This is reflected also in instability of carbohydrate tolerance, which baffles regulation of insulin and diet. In this class belong particularly the 10 cases (A9255 to 1898, inclusive). To define the syndrome further is impossible. The basal metabolism was frequently above normal. In some instances it failed to respond to iodine; in others it changed but little after thyroidectomy. But these were not common characteristics of all cases. For example, under iodine therapy the basal metabolism of A53920 fell from +30 to +16 per cent, suggesting simple hyperthyroidism. Nevertheless, in this case, as in all the others, most of the stigmata of hyperthyroidism were lacking. Moreover, the association of hyperlipemia with hyperthyroidism is

strangely at variance with the claims of Hurxthal (28, 29) and others that serum cholesterol is regularly low in this condition.

Of course, it is conceivable that the hyperlipemia in patients with especially unstable carbohydrate metabolism is referable to a mobilization of fat, similar to that in diabetic acidosis. In both instances carbohydrate metabolism is impaired, the difference being in degree rather than in kind of dysfunction. One argument against this explanation for the hyperlipemia of diabetics with disturbances of the autonomic nervous system is that a non-diabetic patient (98201), with a basal metabolism of +20 per cent 4 months after thyroidectomy and with the same nervous symptoms as A9255 to 1898 (inclusive), had a serum cholesterol of 532 milligrams per cent and total fatty acids of 44.9 milliequivalents. The hyperlipemia here can not be associated with mobilization of fat, contingent upon impairment of carbohydrate metabolism. There is much temptation to place the onus upon the pituitary, since extracts of this gland both diminish carbohydrate tolerance and augment thyroid activity. One case, indeed, had acromegaly. Moreover, in another acromegalic, without any demonstrable disturbances of carbohydrate tolerance or basal metabolism, serum cholesterol of 302 mgm. per cent and total fatty acids of 18.8 m.eq. were discovered. However, in the great majority of cases, it was impossible to implicate the pituitary. In some there were symptoms pointing to affections of the mid-brain (Parkinson's syndrome, diabetes insipidus, etc.), and the nature and degree of autonomic instability in many was suggestive of hypothalamic lesions. It is possible that in the acromegalic cases also the hyperlipemia was referable not directly to the tumor of the pituitary and hyperactivity of the acidophilic cells but to encroachment of these tumors upon subjacent structures in the mid-brain. All this must remain mere speculation until the effects of hypophysis and mid-brain on lipid metabolism are established, either by direct experiment or by studies of more extensive clinical material. An investigation of both diabetic patients and non-diabetic patients with comparable disorders of the autonomic system is now under way.

## CONCLUSIONS

Serum has been analyzed for lipoids in 79 diabetic patients, 20 males and 59 females, who were not suffering from acidosis or dehydration. Cholesterol was normal in 42, below normal in 9, above normal in 28.

Cholesterolemia and phospholipoidemia were closely correlated. The correlation with serum fatty acids was less exact, but gross changes in one component were reflected in the others.

Reasons for the low incidence of hyperlipemia in this series are discussed.

The level of serum cholesterol did not appear to be related to the severity of diabetes, the fat in the diet or the degree of arteriosclerosis.

Hypocholesterolemia was associated with extreme malnutrition and hypoproteinemia.

Mild hypercholesterolemia was observed in obese females, but appeared to be related to the pattern of obesity rather than the diet.

Severe hypercholesterolemia was frequently referable to complicating conditions. It was found also in a group of patients who presented instability of the vasomotor reactions and carbohydrate metabolism. The implications of this observation are discussed.

## PROTOCOLS

*A8513*, male, aged 67, weight 69.5 kgm. Diabetes, cirrhosis of liver (hemachromatosis?), moderate hypertension (140 to 170/70 to 80), heart failure, ascites, edema of legs, bilateral cataracts. Icteric index 6, no retention of bromsulphalein. In addition, there were some reflex changes and a coarse tremor. He proved extraordinarily resistant to insulin and was seldom entirely free from glycosuria. Serum albumin 3.69 to 4.42; globulin 2.36 to 2.81 per cent.

*A10517*, male, aged 51, weight 113 kgm. March 31, 1933, diabetes, slight hypertension (162/84), heart failure, chronic emphysema, mild polycythemia. Diabetes quite mild. April 7, general condition improved. January 10, 1934, recurrence of heart failure and acute bronchitis with exaggerated polycythemia. Blood pressure normal. Liver distinctly enlarged throughout.

*5496*, female, aged 53. Diabetes, malnutrition, hypertension (206 to 176/112 to 100), with profuse albuminuria, secondary anemia and moderate edema, admitted to hospital after a mild cerebral

vascular accident. The last examination was made a few days before death. Throughout her course in hospital she suffered from a psychosis, probably arteriosclerotic in origin. Serum albumin 2.86 to 2.55; globulin 2.52 to 2.70 per cent.

*A1231*, female, aged 54, weight 44.8 to 53.4 kgm. Diabetes, extremely malnourished, with variable amount of edema, profuse albuminuria. hypertension (220 to 140/110 to 60), advanced retinitis, unexplained abdominal pain with vomiting, pulmonary signs suggestive of tuberculosis, but negative sputum. Serum albumin 2.44 to 2.91; globulin 2.39 to 2.07 per cent. Blood non-protein nitrogen 44 to 50 milligrams per cent.

*17383*, female, aged 50, weight 59.4 kgm. Diabetes, hypertension (230/110). One kidney removed in 1920; left hemiplegia in 1930.

*70975*, male, aged 52, weight 81 kgm. Diabetes, chronic glomerulonephritis, with albuminuria, without hypertension. Neoplasm of colon.

*A31776*, female aged 72, weight 59 kgm. Diabetes, arteriosclerosis with gangrene of toe; after mid-thigh amputation, during attack of generalized cutaneous xanthomatosis. At the time of the examination she had persistent tachycardia, profuse perspiration and glycosuria despite large doses of insulin. There were obvious psychic disturbances.

*A9255*, female, aged 61. Diabetes mellitus, acromegaly, bronchial asthma. In addition, there was persistent tachycardia, hypertension, extreme vasomotor instability with frequent profuse sweats, and the carbohydrate metabolism was so unstable that glycosuria could never be eliminated entirely without precipitating hypoglycemic shock. The basal metabolism was plus 79 per cent on January 7, 1933. Subtotal thyroidectomy was performed on February 4, 1933, but had a negligible effect upon the symptoms. Subsequent basal metabolisms were: February 17, + 27; February 24, + 32; March 9, + 27; March 31, + 31; April 25, + 45; June 19, + 35; December 13, + 32; February 6, 1935, + 40. In addition, the pituitary gland was treated by x-ray without appreciable benefit. Signs of increased intracranial pressure were conspicuously lacking throughout.

*A8380*, female, aged 50, weight 48 kgm., wasted. Diabetes, hypertension (175/75), persistent tachycardia, extreme vasomotor instability

with excessive sweating and flushing, chronic urticaria. The carbohydrate metabolism was extremely unstable, making insulin adjustment difficult. It was impossible to obtain a satisfactory basal metabolism because of excessive nervousness; an unsatisfactory determination on October 29 was  $+36$  per cent. There was some symptomatic improvement after iodine therapy.

*A30304*, female, aged 50, weight 44.5 kgm. Diabetes, extremely wasted, with tachycardia, sweating and vasomotor instability, with normal blood pressure. One kidney had been removed in 1932. Glycosuria could not be completely eliminated without precipitating insulin shock. Basal metabolism, May 12,  $+11$  per cent. Serum albumin 3.98, globulin 1.83 per cent.

*A52638*, female, aged 32, weight 47.5 kgm. Diabetes; admitted December 6, 1934, with slight bronchopneumonia and signs suggestive of mitral stenosis. After recovery from the acute infection tachycardia persisted, and the basal metabolism was found to be  $+60$  per cent on December 17. Under iodine it did not fall. Subtotal thyroidectomy was performed on January 15, 1935. On January 29, the basal metabolism was  $+16$  per cent. Regulation of glycosuria was extremely difficult. There were no signs of hyperthyroidism except tachycardia and increased basal metabolism.

*A53920*, male, aged 52, weight 92.5 kgm. Mild diabetes, hypertension (190 to 168/115 to 98), vasomotor instability, excessive perspiration and nervousness. In spite of a basal metabolism of  $+30$  per cent and a greatly restricted diet, he had maintained his weight. The second examination was made after two weeks of iodine therapy when the basal metabolism was  $+16$  per cent.

*69743*, female, aged 52, weight 42.6 kgm. Diabetes, without hypertension, tachycardia or definite signs of hyperthyroidism. She failed to gain weight on a high caloric diet. Glycosuria was regulated with great difficulty. The basal metabolism ranged from  $+33$  to  $+59$  per cent and did not respond to iodine therapy.

*A32114*, female, aged 70, weight 48 kgm. on admission, increasing to 52.4. Diabetes, senile cataracts and general arteriosclerosis without hypertension. Extremely emaciated and dehydrated from starvation diet on admission. She had a

persistent tachycardia, and her carbohydrate metabolism was quite unstable.

*34878*, female, aged 70, weight 57.4 kgm. Diabetes, general arteriosclerosis without hypertension. Parkinson's disease. (This examination was made at 3 P.M.)

*1898*, female, aged 57, weight 64.5 kgm. Mild diabetes, obesity and hypertension (228/116), during recovery from acute follicular tonsillitis. Developed diabetes insipidus about 8 months later.

*A9112*, female, aged 56, weight 57 kgm. Diabetes, mild pernicious anemia, slight tachycardia, unstable carbohydrate metabolism. Basal metabolism — 2 per cent.

*A7358*, female, aged 13, weight 40 kgm. Diabetes, after recovery from acidosis precipitated by an acute exacerbation of a chronic otitis media.

*36671*, female, aged 50, weight 62 kgm. Mild diabetes, moderate obesity, hypertension (198/100), moderate albuminuria, blood nonprotein nitrogen 28 mgm. per cent.

*2854*, female, aged 53, weight 59.5 kgm. Diabetes, moderate obesity, mild hypertension (160/90), burns of toes.

*33222*, female, aged 61, weight 58.1 kgm. Diabetes, moderate obesity, chronic cholelithiasis and cholecystitis, hypertrophic arthritis of spine.

*33580*, female, aged 63 weight 75.9 kgm. Diabetes, obesity, cataracts, blood pressure 210 to 155/95 to 85.

*A9452*, female, aged 48, weight 65.8 kgm. Diabetes, moderate obesity, hypertension (200 to 172/120 to 110).

*A14006*, female, aged 40, weight 76 kgm. Diabetes, obesity. Blood pressure 150 to 126/100 to 86.

*A30212*, female, aged 62, weight 61.1 kgm. Diabetes, arteriosclerosis with hypertension (182/104) and gangrene of toe.

*A31035*, female, aged 57, weight 66.3 kgm. Diabetes, obesity, arteriosclerosis, blood pressure 150/94, ulcer of foot.

*A32422*, female, aged 61. Diabetes, obesity, arteriosclerosis, hypertension (240 to 175/110 to 85), subarachnoid hemorrhage. Lipoid determinations 12 hours before death.

*A53097*, female, aged 59, weight 57.3 kgm. Diabetes, obesity, arteriosclerosis with hypertension (170/90).

29923, female, aged 68. An extremely emaciated diabetic, one day before death from staphylococcus septicemia.

33395, female, aged 55. An extremely emaciated diabetic, one day before death from staphylococcus septicemia. Serum albumin 2.67, globulin 2.02 per cent.

46000, female, aged 65. Diabetes, extreme emaciation, arteriosclerosis with hypertension (170/110) and previous amputation of foot. Serum albumin 2.60, globulin 3.39 per cent.

48983, male, aged 59, weight 62 kgm. (with ascites and edema of legs). Diabetes, cirrhosis of liver, emaciated. Serum albumin 2.47, globulin 3.58 per cent.

49017, female, aged 55. Diabetes, extreme emaciation, arteriosclerosis without hypertension, gangrene of foot, spreading cellulitis, 5 days before death. Serum albumin 3.03, globulin 3.34 per cent.

430909, male, aged 70, weight 49.7 kgm. Diabetes, extreme emaciation, arteriosclerosis without hypertension, multiple ulcers of feet. Serum albumin 3.47, globulin 2.92 per cent.

432215, male, aged 78. Diabetes, extremely wasted, during postoperative recovery from appendicitis and peritonitis. Arteriosclerosis with hypertension (178/90), auricular fibrillation and heart failure, edema. Serum albumin 3.12, globulin 1.75 per cent.

432655, male, aged 71, weight 60.5 kgm. Diabetes, extremely wasted, with duodenal ulcer, bleeding; 8 days before death from acute hemorrhage. Serum albumin 3.43, globulin 1.66 per cent.

432829, male, aged 57. Diabetes, extremely emaciated, with intestinal obstruction. Died January 15. Serum albumin 3.73, globulin 1.28 per cent.

29176, male, aged 46, weight 64.3 kgm. Diabetes, normal nutrition; no known complication.

36052, female, aged 48, weight 81.3 kgm. Diabetes, obesity, arteriosclerosis; blood pressure 160/100.

43494, female, aged 60, weight 55.5 kgm. Diabetes, mild hypertension (150/95).

45815, female, aged 43, weight 57.7 kgm. Diabetes, obesity.

60404, male, aged 14, weight 42.3 kgm. Diabetes, not complicated; slightly obese.

46028, male, aged 50, weight 81.7 kgm. Diabetes, normal nutrition, traumatic ulcer of foot.

414670, female, aged 67, weight 49 kgm. Diabetes, arteriosclerosis. Blood pressure 165/80.

426960, male, aged 19, weight 54.2 kgm. Diabetes, impetigo.

431733, female, aged 55, weight 72.5 kgm. Diabetes, obesity, hypertension (180 to 120/100 to 80).

432622, female, aged 75, weight 50 kgm. Diabetes, normal nutrition, arteriosclerosis without hypertension.

433706, female, aged 25, weight 48.9 kgm. Diabetes, during improvement from malnutrition induced by starvation diet.

436853, male, aged 62, weight 54.3 kgm. Diabetes, ulcer of stomach with pyloric obstruction. During convalescence from gastrectomy. Emaciated, but had taken high caloric diet for some days. Serum albumin 4.41, globulin 2.57 per cent.

442040, female, aged 24, weight 66 kgm. Diabetes.

963, female, aged 54, weight 64.5 kgm. Diabetes, obesity, acute cholelithiasis.

5105, male, aged 50, weight 70.5 kgm. Diabetes, malnutrition, fractures of femur.

18576, female, aged 54, weight 66 kgm. Diabetes, obesity, arteriosclerosis with hypertension (210/110).

29503, female, aged 56, weight 64.2 kgm. Diabetes; admitted with mastoiditis. Later operation for epithelioma of vulva, which resulted in fatal ulceration. The studies were made during the period when nutrition was steadily failing.

34442, female, aged 49, weight 68.2 kgm. Diabetes, obesity, erythema multiforme, arteriosclerosis without hypertension.

36572, female, aged 72, weight 59 kgm. Diabetes, renal calculus.

40992, male, aged 64, weight 78.5 kgm. Diabetes, moderate obesity, arteriosclerotic heart disease, heart failure, blood pressure 120/68.

15096, female, aged 71. Diabetes, poor nutrition, arteriosclerosis with hypertension (195/105), ulcerated abrasion of toe, mild vasomotor instability evidenced by slight tachycardia and sweat-

ing, and carbohydrate tolerance so disturbed that sufficient insulin for glycosuria precipitated hypoglycemic shock.

58487, female, aged 49, weight 81.4 kgm. Diabetes, obesity.

61682, male, aged 57, weight 52.6 kgm. Diabetes, extreme emaciation, arteriosclerosis without hypertension.

79627, female, aged 61, weight 100.2 kgm. Diabetes, extreme obesity, arteriosclerosis with heart disease. During recovery from pneumonia and coronary occlusion, when she had been unable to eat and had lost much weight. Moderate edema.

83790, female, aged 61, weight 67.2 kgm. Diabetes, arteriosclerosis with coronary occlusion, femoral thrombophlebitis and edema.

83896, female, aged 29, weight 67.5 kgm. Diabetes mellitus, chronic pelvic inflammatory disease (gonococcal).

85804, female, aged 58, weight 60 kgm. Diabetes, arteriosclerosis with hypertension (200/100), occlusion of popliteal artery, coronary occlusion.

90339, female, aged 41, weight 66.2 kgm. Diabetes, obesity, gluteal abscess.

94028, female, aged 58. Diabetes, normal nutrition, arteriosclerosis with slight hypertension (162/96), ulcer of foot.

96675, female, aged 52. Diabetes, arteriosclerotic heart disease, coronary occlusion.

A7948, female, aged 71, weight 59.1 kgm. Diabetes, poor nutrition, gastric carcinoma.

A8517, female, aged 56, weight 59.5 kgm. Diabetes, uncomplicated.

A9315, female, aged 67, weight 58.5 kgm. Diabetes, trigeminal neuralgia, cataract, arteriosclerosis with hypertension (152/100).

A15928, male, aged 61. Diabetes, poor nutrition, arteriosclerotic heart disease, heart failure, pleural effusion (possibly due to carcinoma).

A29051, female, aged 51, weight 60.1 kgm. Diabetes, carbuncle of back.

A30054, male, aged 67, weight 86.4 kgm. Diabetes, obesity, mitral stenosis with heart failure, perforating ulcer of foot.

A30790, female, aged 50, weight 68.2 kgm. Diabetes, obesity, non-toxic goitre.

A30940, female, aged 57. Diabetes, multiple

subcutaneous abscesses, emaciation. Serum albumin 2.67 to 3.59, globulin 3.36 to 3.70 per cent.

A32367, female, aged 67. Diabetes, poorly nourished, with arteriosclerosis, coronary disease and gangrene of foot.

A32692, female, aged 66. Diabetes, obesity, gangrene of foot; after mid-thigh amputation.

A33062, female, aged 41, weight 67 kgm. Diabetes, hyperthyroidism, hypertension (190/110). Basal metabolism +41 per cent.

A34635, female, aged 43, weight 56.7 kgm. Acromegaly with mild diabetes; previously treated by x-ray of pituitary.

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metabolism for periods of only one to two and one-half hours after the administration of a protein test meal (8) (9) (10). Since the metabolic increases for the amounts of protein employed in these instances may well be expected to last over six hours (11), one can not conclude as to the total specific dynamic action in these experiments. In two of these investigations (8) (9), the specific dynamic action was expressed as the percentage increase in metabolism observed at one particular determination, at one or at one and one-half hours after the meal. These values give no indication of the total or average metabolic increase. Plaut (12) (13) also observed an increase in metabolism in patients with hypothyroidism during three hours after protein ingestion. The interpretation of the results of these experiments is complicated by the fact that 500 cc. of coffee were included in the test meals (14) (15).

The results and conclusions from *studies of the specific dynamic action of carbohydrate and of protein in animals after thyroidectomy* differ. Baumann and Hunt (16) found that glucose administered to ten rabbits upon which thyroidectomy had been performed resulted in no increase in metabolism or in a smaller increase than was observed after glucose in the same animals preoperatively, or in nonoperated controls. Oxygen and carbon dioxide exchange and total calories were reported over a period of two hours before and over a period of four hours after the glucose was administered. Mansfeld and Scheffer-Csillag (17) conclude that thyroidectomy had no influence on the specific dynamic action of meat or of sugar in two dogs. Hertz (18) denies an influence of thyroidectomy on specific dynamic action of protein in dogs. His conclusions are based upon measurements made at about three and seven hours after feeding meat together with rice and fat to a single thyroidectomized dog. Árvay and Verzá (19) (20) studied the specific dynamic action in thyroidectomized rats over periods of from six to eight hours after feeding meat. They reported a decreased specific dynamic action up to thirty-six days after operation. Dann, Chambers, and Lusk (21) found that their figures in a single experiment incidental to another problem "might suggest that the specific dynamic action of meat is less after thyroidectomy than in the

normal dog. . . ." Houssay and Artundo (22) found that the specific dynamic action, expressed as increase in heat production, measured two and five hours after administering meat to dogs was slightly less after thyroidectomy (6 experiments), and after thyrohypophysectomy (7 experiments), than in controls (10 experiments) but they conclude that the hypophysis and thyroid are not necessary for the production of specific dynamic action.

Due to the usual presence of aberrant thyroid tissue, it is difficult to produce persistent hypothyroidism in animals. In some of the experiments on thyroidectomized animals reported above (16) (17) hypothyroidism as evidenced by a low control metabolic level was not present at all times when specific dynamic action was tested.

#### MATERIALS AND METHODS

Total ablation of the thyroid gland had been performed on the subjects of the present investigation for the treatment of angina pectoris or of rheumatic heart disease (1) (2). Measurements of specific dynamic action were made from two weeks to twenty-one months postoperatively. In two patients experiments were performed preoperatively, one of these patients being tested again two weeks postoperatively and the other two months after operation. Fourteen carbohydrate experiments were performed in eleven patients and five protein experiments in four patients. The metabolism of three of the patients was also measured over a period of five hours without food. The average basal metabolic rate at the time of the postoperative measurements of specific dynamic action was minus 28 per cent. The subjects were from eighteen to sixty-five years of age. All of the patients were maintaining a practically constant body weight; one was obese (E. P.), the others were well developed. Signs or symptoms of congestive heart failure were not present at the time of the tests, nor were anginal attacks experienced just prior to or during any of the experiments. The subjects of eight experiments were taking thyroid (grains  $\frac{1}{10}$  to  $\frac{1}{2}$  daily, Armour) to maintain them free from discomforting symptoms of myxedema; and one patient (M. S.) had 150 mgm. of dinitrophenol on the day prior to the test.

All subjects were well trained in metabolism tests. They came to the laboratory from home or from the hospital wards in the morning in the postabsorptive state, without medication, and in some cases having omitted thyroid on the preceding day. After the patient had rested at least thirty minutes either flat in bed or slightly raised, a determination of oxygen consumption was made for a period of seven minutes using a Collins Benedict-Roth apparatus. Three to five such measurements (usually four, consisting of two determinations in duplicate)

were made over about one hour in order to obtain a control period; following this, the carbohydrate or protein meal was administered.

In the carbohydrate tests fifty to seventy-five grams of glucose were ingested dissolved in 200 grams of orange juice and heated to approximately 36° C. The meals totalled 280 to 375 calories (the caloric value of 1 gram of hexose is 3.74 calories). Measurements of oxygen consumption were begun fifteen minutes after the ingestion and continued, at intervals, for two to three and one-half hours, a total of six to nine measurements being made after the meal. When duplicate measurements did not check or seemed erratic, additional measurements were made. The results obtained were plotted noting each observation upon the graph. From smoothed curves through the points obtained the oxygen consumption at  $\frac{1}{4}$ ,  $\frac{1}{2}$ , 1,  $1\frac{1}{2}$ , and 2 hours were tabulated.

In the protein tests from 200 to 400 grams of steak, with small amounts of added protein, fat, and carbohydrate (54 to 102 grams P, 25 to 44 grams F, 6 to 23 grams CHO = 520 to 860 calories) were ingested after the control level of metabolism was determined. The average protein intake was 77 grams, the average total calories 685. The oxygen consumption was followed at

hour after smoking are not held trustworthy (23). If the measurements following the smoking be omitted in drawing the course of the oxygen consumption, the actual points thirty minutes after smoking are 0, 0, 6, and 5 per cent above the curves thus obtained.

The procedures were not discomforting but not enjoyable, and in three cases the subjects asked as to how soon the test would be finished. Only one subject (E. P.) could be considered uncooperative. A tendency to drowsiness was almost universal, but most of the subjects remained quietly awake. The more intelligent patients were told something of the nature of the test; others merely followed instructions. The low metabolic rates, experience with the apparatus, and almost universal willingness to cooperate, all helped to maintain the subjects at ease.

## RESULTS

### *Specific dynamic action of glucose*

The results of eleven experiments on nine patients, two to twenty-one months after total thyroidectomy, are presented in Table I. Two stud-

TABLE I  
*Specific dynamic action of carbohydrate after total thyroidectomy*

Case	Age	Diagnosis†	Months post-operative	Basal metabolic rate. Deviation from normal	Carbo-hydrate meal	Oxygen consumption												Thyroid per day	
						Basal	Hours after test meal												
							¼		½		1		1½		2				
								In-crease		In-crease		In-crease		In-crease		In-crease			
	years		per cent	calories	cc. per minute	cc. per minute	per cent	cc. per minute	per cent	cc. per minute	per cent	cc. per minute	per cent	cc. per minute	per cent	grains			
E.P.	58	A.P.	7	-28	280	150	153	2.0	155	3.3	155	3.3	152	1.3	153	2	1/2		
M.S.	46	A.P.	3½	-21	280	173	189	9.3	185	7.0	177	2.4	182	5.2	181	4.7	1/4		
M.S.	46	A.P.	3½	-26	375	163	176	8.0	168	3.1	159	-2.3	156	-4.2	156	-4.2	dnp*		
H.W.	34	R.H.D.	3	-30	375	170	186	9.4	186	9.4	184	8.2	170	0	162	-4.6	0		
H.W.	34	R.H.D.	3½	-37	375	152	162	6.6	168	10.6	172	13.2	169	11.2	168	10.6	0		
H.K.	61	A.P.	4½	-34	280	156	161	3.2	167	7.1	170	9.9	174	11.6	(170)	8.9	1/8		
H.G.	24	R.H.D.	12	-37	375	156.5	166	6.1	168	7.3	170	8.6	171	9.3	172	9.9	3/20		
F.F.	36	R.H.D.	2	-21	375	164	177	7.9	186	13.4	186	13.4	181	10.3	179	9.2	0		
F.D.	19	R.H.D.	13	-37	375	153	176	15.1	168	9.8	158	3.3	159	3.9	163	6.6	0		
T.C.	65	A.P.	10	-40	375	149	160	7.3	159	6.7	162	8.7	157	5.3	148	-0.6	3/20		
G.F.	54	R.H.D.	21	-25	375	175	192	9.7	186	6.3	184	5.1	178	1.7	175	0.0	0		
Average:				-30.5	350	160.1	173.5	7.7	172.4	7.6	170.7	6.7	168.1	5.1	165.1	3.9			

\* 150 mgm. dinitrophenol on day preceding test.

† Diagnoses: A.P. = Angina pectoris; R.H.D. = Rheumatic heart disease.

intervals for six to seven hours afterwards, measurements being made in duplicate, and the results of each measurement were plotted. One subject had three puffs on a cigar, another had a cigarette directly after the meal; two subjects each had a cigarette five hours after the meal. Not knowing whether smoking or its denial would vitiate results more, the effort was made to keep the patient at ease. Measurements made within a half

ies are not included because of our inability to obtain check measurements. Another experiment was performed only two weeks after operation and is referred to later. The oxygen consumption per minute at one-fourth, one-half, one, one and a half, and two hours were obtained as described above. The percentage increase in oxy-



gen consumption over the basal at each point has also been calculated (Table I). The averages of the values for oxygen consumption in Table I are plotted as the lower curve in Figure 1. The total

The average basal metabolic rate at the time of the glucose experiments was minus 30.5 per cent, the range was from minus 21 to minus 40 per cent (Table I). The maximal increase in oxygen

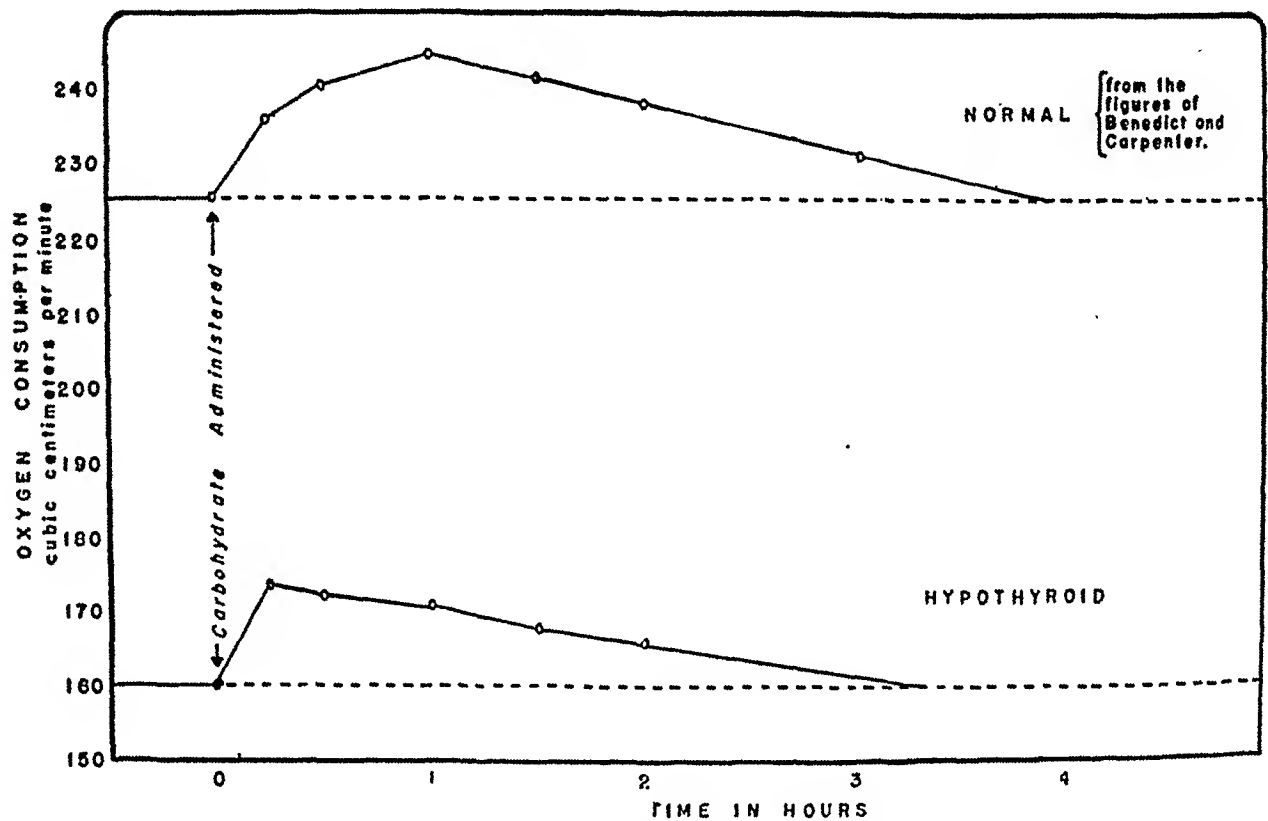


FIG. 1. CHANGE IN RATE OF OXYGEN CONSUMPTION AFTER THE INGESTION OF 75 TO 100 GRAMS OF CARBOHYDRATE.

*Lower curve.* Average of eleven experiments upon patients with hypothyroidism. *Upper curve.* Average of twelve experiments by Benedict and Carpenter (11) upon normal individuals.

increases in oxygen consumption or the areas under the curves above the control levels have been calculated for the arbitrary period of two hours and for the actual or extrapolated duration of the increases. The average, the maximal, and the minimal increases and the corresponding percentage increases are listed in Table II.

consumption after the glucose in the different experiments ranged from 3.3 to 15.1 per cent of the basal oxygen consumption (average 10.1 per cent). This maximal increase occurred at one-quarter hour after the test meal in five cases, one-half hour in two cases, one hour in two cases, one and one-half hours and two hours in one case

TABLE II  
Summary of specific dynamic action after carbohydrate ingestion

	Carbo- hydrate meal	Basal meta- bolic rate. Deviation from normal	Basal oxygen consump- tion	Maximum oxygen consumption		Increase in oxy- gen consumption for two hours		Total increase in oxygen consumption		Extra calories (R.Q. 0.88)	"Cost" (extra calories per 100 calories ingested)
	calories	per cent	cc. per minute	cc. per minute	increase per cent	cc.	per cent	cc.	per cent		
Average.....	350	-30.5	160.1	174	8.75	1160	6.0	1400	4.4	6.8	1.9
Maximum.....	375	-21	164	188	14.64	2138	10.8	3300	7.4	15.9	4.2
Minimum *....	280	-28	150	156.5	4.33	540	4.5	780	3.3	3.8	1.4

\* Experiment 1 (E.P.) in Table I—(From Table I; experiment 3 (M.S.) gives a smaller increase, but due to apparent negative values this finding is questionable).

each. The maximal increase of the average curve was 8.7 per cent, the increase over two hours was 1160 cc. or 6 per cent; the total increase was 1400 cc. or 4.4 per cent (over 3 and  $\frac{1}{3}$  hours). Assuming an average R.Q. of 0.875<sup>2</sup> after the food there were 6.8 extra calories produced or 1.9 calories for every 100 calories from carbohydrate ingested. The results of experiments in two subjects performed both before and after operation are presented in Table III. In both of these sub-

### *Control measurements of metabolism without test meal*

The oxygen consumption in Patient G. F. fluctuated during four hours by  $\pm 2.7$  per cent from a level of 165 cc. of oxygen per minute. Four weeks previously during the one hour control period before a protein meal the oxygen consumption decreased from 171 cc. to 167 cc. per minute. The oxygen consumption in Patient W. D. decreased progressively during four hours

TABLE III  
*Summary of specific dynamic action of carbohydrate in subjects studied both before and after operation*

	Basal metabolic rate. Deviation from normal	Carbohydrate meal	Maximum increase in oxygen consumption		Increase in oxygen consumption over two hours		Total increase in oxygen consumption		"Cost" (extra calories per 100 calories ingested)
	per cent	calories	cc. per minute	per cent	cc.	per cent	cc.	per cent	
R.S.—preoperative.....	+ 8	280	30	11.5	2160	7	2160	7	3.8
2 weeks postoperative *.....	-13	375	52	24	3960	15	4010	14	5.2
F.F.—preoperative.....	- 9	375	12	6.3	1200	5.3	1440	4.3	1.9
2 months postoperative.....	-21	375	22	13.4	2180	11.1	3284	7.3	4.3

\* This experiment was not included in Table I or Table II., because only two weeks had elapsed since operation.

jects the specific dynamic action was higher in the experiments after operation than before.

### *Specific dynamic action of protein*

The results in five experiments on the four patients are summarized in Table IV. One-half hour after the administration of the protein test meal a rise in oxygen consumption was observed. The oxygen consumption reached its average maximum between one-half and three hours after the meal and remained at about that level for the duration of the experiment (6 to  $7\frac{1}{2}$  hours). From the curves plotted as described the average total increase during the experiments was 10,570 cc., equivalent to approximately fifty extra calories or to an average increase of 16.6 per cent over the basal oxygen consumption. The caloric increase was 7 per cent of the caloric intake or 17 per cent of the protein calories ingested. The average basal metabolic rate for this series was minus 28.6 per cent.

<sup>2</sup> The average respiratory quotient from 0 to 3 hours after dextrose in the experiments of Benedict and Carpenter was 0.875 (11).

from 182 to 167 cc. per minute. In an experimental control one week later the oxygen consumption decreased from 187 to 180 cc. per minute in one hour and again two weeks later from 185 to 182 cc. per minute in one hour. The oxygen consumption in Patient F. D. during the first hour was 130 cc. per minute  $\pm 15$  cc. (11.5 per cent). From then through the fourth hour the oxygen consumption remained 130 cc.  $\pm 5$  cc. (3.8 per cent). In an experimental control one week previously the oxygen consumption was 145 cc. per minute  $\pm 2$  cc. (1.4 per cent) over three-quarters of an hour.

### DISCUSSION

The purpose of the above study was to discover whether the specific dynamic action of carbohydrate and of protein in patients with hypothyroidism following total thyroidectomy differed significantly from that reported for normal individuals. The method of studying specific dynamic action which has been utilized above was chosen because of its simplicity and because early experiences showed it to be suitable to our pur-

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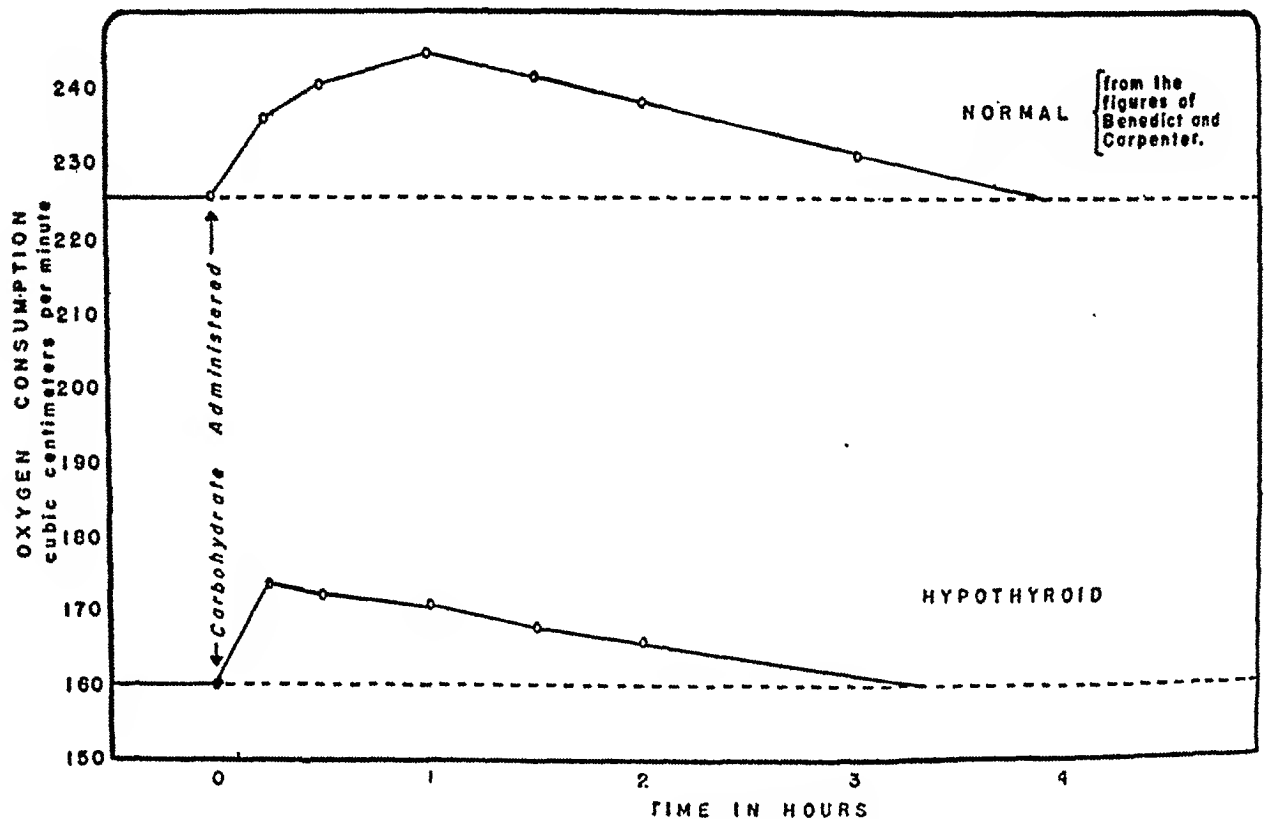


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<sup>2</sup> The average respiratory quotient from 0 to 3 hours after dextrose in the experiments of Benedict and Carpenter was 0.875 (11).

TABLE IV  
Summary of specific dynamic action after protein ingestion

Case	Age	Diagnosis	Months post-operative	Test meal		Basal metabolic rate. Deviation from normal	Oxygen consumption		Maximum increase over basal		Time to reach maximum	Duration of approximate maximum increase	Total increase in oxygen consumption		Increase in oxygen per 100 grams protein		Thyroid per day
				Protein	Total calories		Basal	Approximate maximum	Total	Per 100 grams protein							
	years			grams		per cent	cc. per minute	cc. per minute	per cent	per cent	hours	hours	cc.	per cent	cc.	per cent	grains
G.F.	53	R.H.D.	12	102	860	-27	168	208	24	24	2	7½	11,240	17	11,030	16	0
W.D.	23	R.H.D.	9	54	520	-25	180-170*	198	16	30	2 ¾	6	10,440	16	19,350	30	1/8
W.D.	23	R.H.D.	9	56	600	-21	180-170*	190	12	21	2 ½	7½	8,400	11	14,850	20	1/8
F.D.	18	R.H.D.	4	70	620	-38	145	168	16	23	3	7	7,475	14	10,500	20	1/3
S.F.	53	A.P.	10	102	825	-32	159	212	33	33	2 ½	6	14,300	25	14,100	25	1/10
Average				77	685	-28.6	166	195	20	26			10,570	17	14,000	22	

\* During a control experiment without food the oxygen consumption of this patient fell from 182 to 167 cc. in four hours. The experimental controls over one hour before the test meals likewise showed a slight progressive fall in oxygen consumption (see text).

pose. In the well trained subjects in this investigation the error with which the oxygen consumption over six minutes could be measured by a Benedict-Roth apparatus was 3 per cent or less. Obviously, the results over the entire experimental period are not as accurate as could be obtained by continuous calorimetry, but by the use of duplicate and serial determinations they are sufficiently precise to render the interpretations valid.

It would have been theoretically more desirable to use a group of patients who were not taking any thyroid, and whose metabolic rates were even lower and symptoms of myxedema even more pronounced than in the present series. No consistent difference in specific dynamic action could be found, however, between the results in those patients who were taking thyroid (grains ½ to 1½ daily) and in the patients to whom no thyroid was being given. In addition, the nature of the response of oxygen consumption after the administration of sugar was not correlated with the basal oxygen consumption or the "basal metabolic rate."

A consistent increase in oxygen consumption occurred in all experiments (Table I) following the administration of carbohydrate. The increase in oxygen consumption is interpreted as resulting from the ingestion of carbohydrate, for the amount of fluid administered was insufficient to affect the metabolism (11) and in many cases a return to basal oxygen level occurred within two to three hours after the meal.

Many methods of expressing specific dynamic action have been utilized. The data of our experiments allow calculation in absolute and percentage values on the basis of maximal oxygen increase, total and average increase over two hours, and over the extrapolated duration of the increase; in the carbohydrate tests the results may be expressed in addition, as total extra calories, and extra calories per hundred grams of carbohydrate, by assuming a respiratory quotient of 0.88 with an error of  $\pm 2.5$  per cent.

Our data calculated in these several ways are compared with similarly treated values for oxygen consumption from the experiments of Benedict and Carpenter (11) and of Gephart and DuBois (24) for the specific dynamic action of glucose in subjects with normal metabolic levels (Table V). Previous to operation, not only were most patients insufficiently trained in the technique of metabolism testing but their clinical conditions would not permit of satisfactory determinations of specific dynamic action. It was felt, therefore, that the results of other authors using well trained subjects with normal metabolic levels would serve as a more accurate basis for comparisons with the present series. From Benedict and Carpenter's work (11) are taken nine experiments in which 100 grams of glucose were given and three experiments in which 75 grams were given; in all, twelve experiments on ten subjects. From Gephart and DuBois (24) six experiments upon three patients are reported. The average maximal in-

TABLE V  
Comparison of specific dynamic action of carbohydrate in normals and thyroidectomized patients

Comparison of specific dynamic action of carbohydrate and protein

Series	Num-ber of sub-jects	Num-ber of experi-ments	range average	Basal oxygen consumption	Basal metabolic rate, Deviation from normal	Test meal, average calories	Oxygen consumption						Extra calories	"Cost" (Extra calories per 100 calories ingested)
							Average of maximum increases	Maxi-mum increase of aver-age of experi-ments	Two hour increase		Total increase			
									cc. per minute	per cent	cc.	per cent		
Present hypothyroid . . . . .	9	11	149-175 160	-21 to -40 -30.5	280-375 350	5-23 16	3.3-15.1 10.1	1.1 8.7	540-2138 1160	4.5-10.8 6.0	780-3300 1400	3.3-7.4 4.4	3.8-15.9 6.8	1.4-1.2 1.9
Normal [Benedict and Car-penter (11)] . . . . .	10	12	average	normal	358	23.2	10.1	20	1575	5.8	2373	5.1	11.5	3.2
Normal [Gephart and Du-Bois (24)] . . . . .	3	6	average	normal	660				2640	8.6	4230	6.5	20.4	3.1

crease, the two hour increment, and the total increment in oxygen consumption after carbohydrate in the present series is less than in the control series of normals but the range of results overlaps. The average percentage maximal increase and percentage two hourly increase are essentially the same in both the normal and thyroidectomized subjects. The average percentage increase over the entire duration of the increase is approximately the same in our series as in the series of Benedict and Carpenter (11), but is slightly less as compared to the figures of Gephart and DuBois (24) who administered more sugar (Table V). Calculated as extra calories expended for 100 calories of food (assuming an R.Q. of 0.88) the average specific dynamic action in our patients is somewhat less than in both series of the controls, but again the results in individual instances overlap. The carbohydrate experiments performed on two of our patients both before and after operation show in each case a greater specific dynamic action following thyroidectomy (Table III). It is to be noted, however, that these postoperative experiments were done only two weeks and two months after operation. The basal metabolic rates of the two patients with hypothyroidism on whom specific dynamic action studies after carbohydrate were made by DuBois (5) and by Weisz and Adler (6) were only reduced to minus 20 per cent (5) and minus 15 per cent (6) at the time of the tests. In these instances, as in our patients with lower basal metabolic rates, the results obtained were not different from those obtained in some normal individuals.

There is no apparent statistical correlation between the basal rates of oxygen consumption and the total increase in oxygen consumption per 100 grams of ingested sugar as calculated from the combined experiments of Weisz and Adler (6), Benedict and Carpenter (11) Gephart and DuBois (24) and the present series. The coefficient of correlation of these thirty-eight determinations of specific dynamic action in normals and hypothyroids is plus .2 (a perfect correlation would be either plus or minus 1.00, absence of correlation is indicated by 0).

The increase in oxygen consumption after the ingestion of from 54 to 102 grams (average 77 grams) of protein in five experiments on four patients with hypothyroidism is similar to the in-

crease in oxygen consumption after protein (average 90 grams, range 45 to 223 grams) in 16 experiments on 9 normal individuals reported by Benedict and Carpenter (11) (Table VI, *a*).

These results are interpreted as demonstrating the presence of specific dynamic action of hexose in human hypothyroidism following total ablation of the normal thyroid gland. Although this spe-

TABLE VI  
*Comparison of specific dynamic action of protein in normals and in thyroidectomized patients*

Series	Number of subjects	Number of experiments		Protein intake	Duration of measurement	Oxygen consumption					
						Basal	Increase after food				
							Average increase		Total increase	Increase per 100 grams protein	
				grams	hours	cc. per minute	cc. per minute	per cent	cc.	cc.	per cent
Present hypothyroid...	4	5	range	54-102	6-7½	145-180	19-40	11.3-25	7475-14,300	10,600-19,350	16-30
			average	77		166.4	27	16.6	10,570	14,000	22.2
Normal [Benedict and Carpenter (11)]	9	16	range	45-223	4½-7	218-295	10.5-69	4-27	3850-18,900	5,640-30,500	11.5-35
			average	90		258	38	15.3	12,950	14,700	18.3
	(b).....	13	range	43-111	2-12	193-260	14-57	6-25	2700-28,500	2,970-40,000	
			average	60		234	34	14.4	9400	15,600	

The increase in our experiments is also similar to that as estimated from the caloric increases reported in another series of thirteen experiments upon nine normal subjects by Benedict and Carpenter (11) (Table VI, *b*). There is considerable variation in the specific dynamic action of protein calculated in several different manners (Table VI), both among normal subjects and among our patients with hypothyroidism.

#### SUMMARY AND CONCLUSIONS

1. The specific dynamic action after the ingestion of 70 to 95 grams of carbohydrate was studied in eleven instances on nine patients at times varying from two to twenty-one months after total thyroidectomy. The average basal metabolic rate at the time of the eleven tests was minus 30.5 per cent. The oxygen consumption increased in each instance after the carbohydrate meal, the increase lasting from two to four hours. The average of the maximal increases over the basal oxygen requirements was 16 cc. of oxygen per minute or 10.1 per cent, and the average total increase was 1400 cc. per minute or 4.4 per cent for the average duration of the experiments (3 and ½ hours).

cific dynamic action appears in percentage to be approximately equal to, it is, on an absolute basis, somewhat less than the average of figures for normals taken from the literature.

2. The specific dynamic action after ingestion of 54 to 102 grams of protein was studied in five instances on four patients, at times varying from four to twelve months after total thyroidectomy. The average basal metabolic rate at the time of the five tests was minus 28.6 per cent. The oxygen consumption increased in each instance after the protein meal, the increase lasting at least six to seven hours. The average maximal increase was 29 cc. per minute (20 per cent of the basal oxygen requirement) and the total increase over the observed period (6 to 7 hours) was 10,570 cc. (16.6 per cent of the basal oxygen requirement) or 14,000 cc. per 100 grams of protein. These results are interpreted as indicating the presence of a specific dynamic action of protein in the totally thyroidectomized hypothyroid human subject. The extent of the specific dynamic action is within the range of normal as compared with values found in the literature.

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# THE INFLUENCE OF VARYING A<sub>s</sub>-V<sub>s</sub> INTERVALS ON SPLIT FIRST HEART SOUNDS: ITS BEARING ON THE CAUSE OF SPLIT SOUNDS AND THE MECHANISM OF THE FIRST SOUND<sup>1</sup>

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(Received for publication May 15, 1935)

It has long been recognized that the first heart sound may include two major components (1). Under certain circumstances these two components may be found on auscultation to be separated so that the sound becomes split or reduplicated, a phenomenon that can be recorded by methods for registering heart sounds. Splitting of the first sound is present in a certain proportion of patients with either left or right complete bundle branch block (2) and in some with lesser grades of intraventricular conduction defect. (Unpublished observations, Wolferth and Margolies.) It is also present in many individuals with presumably healthy hearts (3). There tends to be a phasic respiratory incidence in the width of the split which is particularly marked in the presumably healthy individuals. Thus, in some cases, if the heart sounds are recorded throughout cycles of respiration, the first sound may be found split during certain phases of the cycle, and single during certain other phases (Figure 1). With an adequately damped apparatus for registration, the split components are recorded as a series of vibrations of short duration. In a case with phasic incidence of splitting the single first sounds are nearly always of considerably longer duration than either component of split sounds. Furthermore, if the duration of either component of a split sound is compared with the duration of the first sound in a case which shows no splitting, the duration of the latter is usually greater. This would suggest that even when there is no determinable splitting of the first sound both components may be present and at least partially merged.

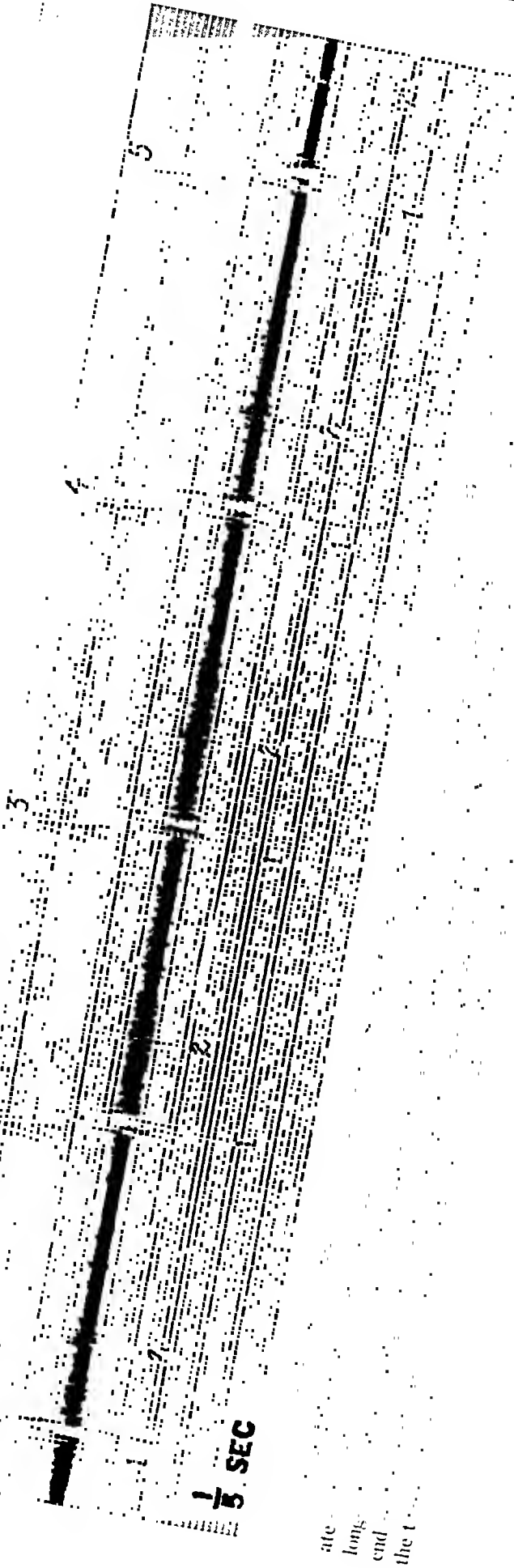
The first step in the study of the mechanism of the first sound should be an attempt to discover what causes this duality of the sound. One gets

but little help from the literature. Some writers have maintained that in split sounds, one component arises in the left and the other in the right ventricle (4). Others have contended that one component is muscular and the other valvular in origin. Thus Wiggers (5) states: "It is generally accepted . . . that the first sound is fundamentally due to vibrations arising from: (a) the friction of the interlacing bands of contracting ventricular muscle, and (b) the closure of the A-V valves." He comments, however, that such an explanation accounts only for the main crescendo vibrations and leaves out of consideration both the introductory and final vibrations of the first sound and does not account for the second components of the aortic first sound. He found that vibrations recorded directly from different regions of the right and left ventricles show no essential differences. Hence, he states, they may be referred to in common as the ventricular sounds.

Recently, in the course of a study of time relations of various events dependent on right and left ventricular contraction, undertaken in the effort to discover the side of the significant lesion in the common type of bundle branch block (6), we have obtained some data that bear on the mechanism of split first sounds. In the few cases studied with widely split first sounds, asynchronism was found in ejection from the right and left ventricles, which corresponded roughly to the degree of asynchronism between the two major components of the sound.<sup>2</sup> Furthermore, the studies indicated that in these cases with widely split first sounds, the first component began before ejection from either ventricle had begun and

<sup>1</sup> Presented in abstract before the American Society for Clinical Investigation May 6, 1935.

<sup>2</sup> Asynchronism in ejection from the two ventricles had previously been noted by Katz (7) in an experimental study on dogs. No attempt, however, was made to correlate this finding with the heart sounds.



... interval from the beginning of the major vibrations in beat 2 is due to close approximation but not synchronism of

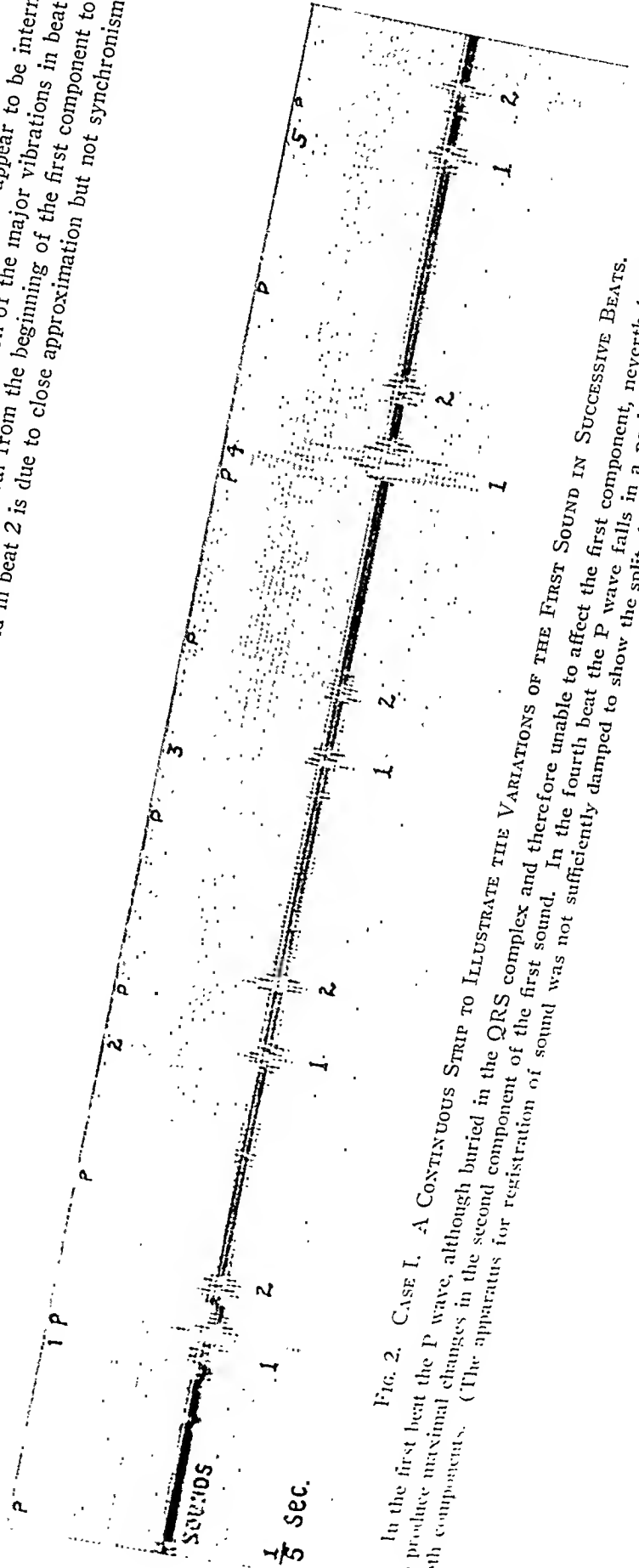


FIG. 2. CASE I. A CONTINUOUS STRIP TO ILLUSTRATE THE VARIATIONS OF THE FIRST SOUND IN SUCCESSIVE BEATS. In the first beat the P wave, although buried in the QRS complex and therefore unable to affect the first component, nevertheless falls early enough to produce maximal changes in the second component of the first sound. In the fourth beat the P wave falls in a position where it is able to influence both components. (The apparatus for registration of sound was not sufficiently damped to show the split clearly in all beats.)

the second component usually after ejection from one ventricle had begun but always before ejection from the other.<sup>3</sup> These correlations were regarded as pointing toward the hypothesis that, at least in certain cases, one of the major components of sound was contributed by the left ventricle and the other by the right ventricle. The significance of the evidence was discussed in the paper referred to (6).

The methods used to time the beginning of right and left ventricular ejection are accurate enough to reveal the presence of significant asynchronism in cases with widely split first sounds. However, in cases with slight splitting or merely prolongation of the first sound the possible errors in the measurements are sufficient to render the results of doubtful value. In view of the importance of establishing the nature of the duality of the first sound it seemed desirable to find some other method of testing the validity of the hypothesis.

It has long been known that in patients with complete heart block there may be marked variation in the loudness of the first sound from beat to beat. Griffith (9), who was the first to point out this phenomenon, concluded that the loud beats tended to occur when the auricles and ventricles beat at about the same time. Several years ago we made a study of this phenomenon and found that the As-Vs time relationship (as measured by the P-R relation) exerts a remarkable influence on the amplitude of recorded vibrations of the first heart sound not only in complete heart block but also in other conditions (10). Furthermore, it could be demonstrated that the marked variations in amplitude corresponded to variations in loudness of the sounds recognizable by auscultation. Slight changes in As-Vs time were sometimes found to be associated with marked variations in the first sound. As a matter of fact, among the various factors governing loudness of

the first sound in cases with normal mechanism the As-Vs time interval is one of the most important. (Margolies, unpublished observations.) Other conditions remaining the same, cases with short normal P-R intervals (0.12 to 0.14 second) tend to have sharp, loud first sounds, whereas cases with long normal P-R intervals (0.18 to 0.21 second) tend to have dull, faint first sounds.

In two cases of complete heart block with split first sounds it was noted that the recorded vibrations of the two components during certain ranges of P-R relation varied in amplitude independently of each other. In one case with varying P-R intervals, due to ventricular escape, similarly marked variations in the two components of split sounds have been observed. In a case with complete heart block and a prolonged but not split first sound, variations were noted in the early and late parts of the prolonged sounds dependent on the As-Vs relationship. It is the purpose of this paper to present data obtained in these cases and to discuss their bearing on the nature of the duality of the first sound.

*Case I.* J. H., an aged inmate of the Moss Home of the Jewish Hospital, was found to have complete heart block, an intraventricular conduction defect (shown by the QRS complex having a duration of 0.14 second), widely split first heart sounds and varying loudness of the first sound from beat to beat. The relative contributions of the two components to the variations in loudness of the first sound could not be recognized with any degree of accuracy on auscultation.

In Figure 2, a continuous strip of tracing is reproduced illustrating (a) the variations in amplitude of vibrations of the first sound in successive beats and (b) the two positions at which major vibrations may occur. In Figure 3, representative beats have been selected from a continuous strip (except beat 5 which was selected from another tracing) and arranged in an order to show the influence of various As-Vs relationships on the sound components.

Analysis of the variations in amplitude of the major vibrations of the two sound components shows the following points: 1. The variations are great, in this respect corresponding to marked variations in intensity of the sounds as determined by auscultation. 2. The variations in the two

<sup>3</sup> Wiggers (8) states that the main vibrations of the first sound begin precisely with the onset of the rise of pressure within the ventricles and on the descending limb of the R-2 wave of the electrocardiogram, and reach their maximal amplitude during the isometric period. Our findings, however, indicate that in cases with split first sounds, the isometric contraction periods in the two ventricles are asynchronous and that the two ventricles, therefore, contribute main vibrations at different times.

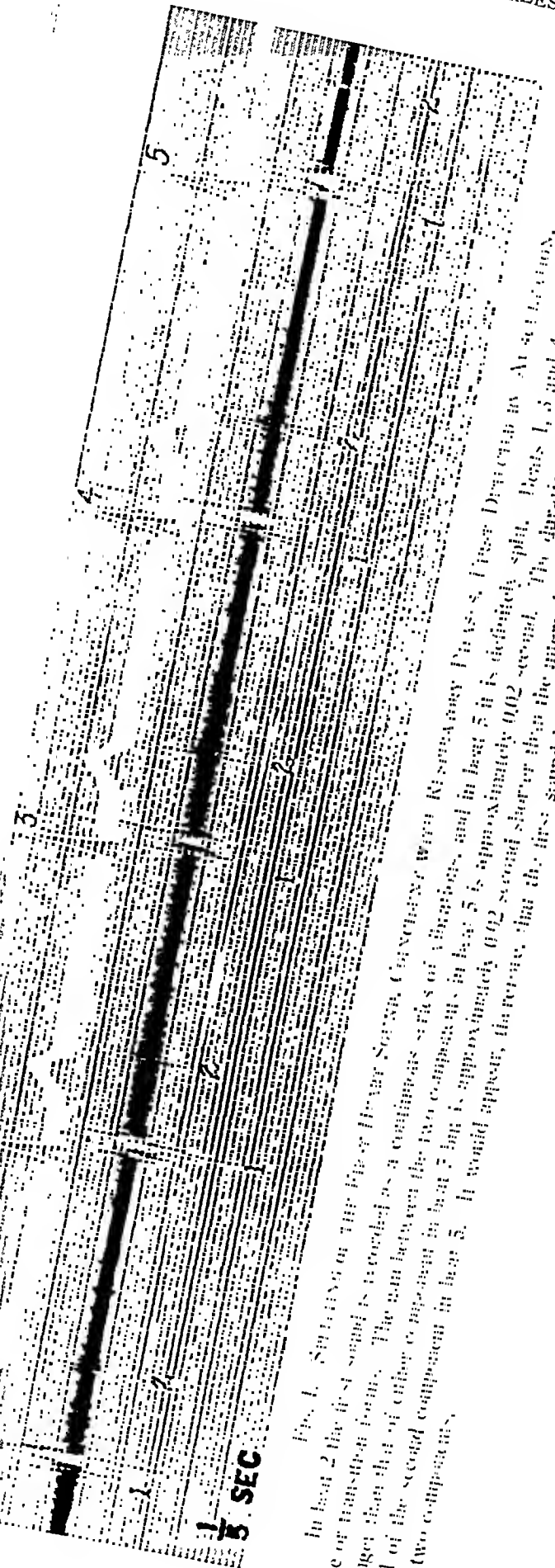


FIG. 1. STRIP RECORDING OF THE P-WAVE COMPONENT WITH VARIATIONS IN P-WAVE DURATION. In beat 2 the first sound is recorded as a continuous series of vibrations, and in beat 5 it is definitely split. Beats 1, 3 and 4 appear to be intermediate of the two components. The duration of the major vibrations in beat 2 is approximately 0.02 second. The duration of the first component to the end of the second component in beat 5 is approximately 0.02 second shorter than the interval from the beginning of the first component to the end of the two components. It would appear, therefore, that the first sound in beat 2 is due to close approximation but not synchronism of

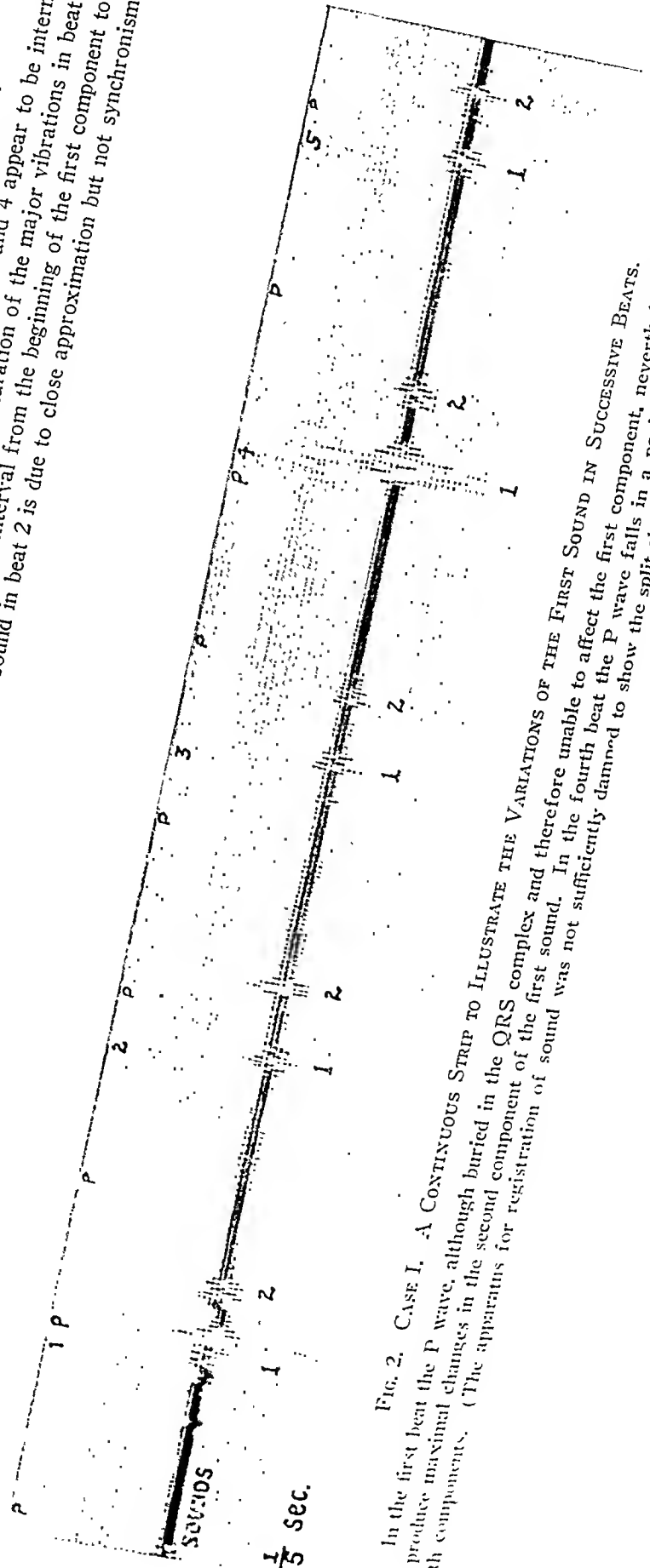


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components are, to a certain extent, independent of each other. The following combinations occur:—both components may be recorded as having (a) small vibrations, (b) large vibrations, (c) the first component may have vibrations of large or intermediate size and the second component small vibrations, (d) the first component may have small vibrations and the second component vibrations of large or intermediate size. Thus practically all combinations of large and small vibrations may occur. 3. The variations cannot be attributed to respiration since (a) there is no correlation with the respiratory cycle and (b) variations continue when the breath is held.

The question must be considered as to whether the behavior of the two sound components described above could occur if both components of sound originated in the same ventricle. There are at least two reasons making such an explanation improbable.

1. Our previous work (6) showed that the first sound component falls during the isometric contraction phase of one ventricle. Thus, in Case I, if both components originated in the same ventricle, the second component, which falls 0.10 second after the first component, would have to occur well along in the course of ventricular contraction. Although under such circumstances it is quite conceivable that the second component might show variations dependent on As-Vs intervals, due to the effect of auricular activity on ventricular contraction, it is unreasonable to suppose that auricular contraction could influence the early part of ventricular contraction in one way and a later part of ventricular contraction in another way. In a discussion as to the various ways in which auricular contraction might influence ventricular behavior and consequently modify the first sound, we have pointed out that three factors might be involved: (a) ventricular filling, (b) ventricular pressure and (c) position of the A-V valves (11). The time at which these factors may exert an effect must be at about the beginning of ventricular contraction. Thus, it seems necessary to assume, other phenomena remaining constant, that the character of a ventricular response must be determined at or before the time the first component of sound occurs. Under these circumstances it is theoretically pos-

sible that the second component might vary (a) in the same direction as the first component, (b) in the opposite direction or (c) remain relatively constant. However, no rational explanation of independent variations based on the postulate that both components arise in the same ventricle occurs to us.

2. It was noted that changes in the second component of sound occurred when auricular contraction fell so near the first component that it could scarcely have had a material influence upon the first component. Thus, in both Cases I and II (the latter to be reported below) auricular activity had no effect on the first component of sound when the P wave was buried in the QRS complex, although the second component in certain beats was materially influenced. As a matter of fact, in Case I the vibrations of the first component were not increased until the P-R relation exceeded 0.03 second, and in Case II, not until the P-R relation exceeded 0.05 second. In connection with these extremely short P-R relations maximal amplitude of the vibrations of the second component occurred. In certain of the beats, in which only the vibrations of the second component were significantly increased, the P wave fell so late that auricular contraction could not have influenced the first component. Thus, in Figure 5, beat 7 (Case II) the P wave is completely buried in the QRS complex. Since the first sound component began 0.04 second after the beginning of the QRS complex, the beginning of the P wave could not have preceded the first component by more than 0.04 second. Taking into account the latent period of auricular muscle and the fact that ventricular contraction must have begun before sounds of ventricular origin could be recorded from the chest wall, it would appear that insufficient time was available for auricular contraction to have influenced the first component. As a matter of fact, apex cardiograms in this case showed no evidence of auricular activity until 0.06 to 0.07 after the beginning of the P wave; the summit of the wave due to auricular contraction fell 0.15 second after the beginning of the P wave. The summit of this wave usually falls 0.08 to 0.14 second after the beginning of the P wave (12).

The above mentioned facts led us to reject the view that both main components of sound could



have originated in one ventricle. Moreover, because of the evidence previously obtained suggesting that each ventricle contributes a component to split first heart sounds (based on the association of split first sounds and asynchronism in ejection), it seemed important to consider whether the variations in the two sound components were in accord with such a postulate.

It seemed to us that independent variation in the two sound components must be accounted for by equally independent effects of some factor or factors which influence production of sound. As indicated above, it is obvious on comparison of the variations of the two sound components with the duration of the P-R interval that some A-V relationship must be responsible for the variations in sound. The P-R interval, however, is a measurement of auriculoventricular conduction and, although it may serve to a certain extent as an indicator of the time relation of beginning auricular and ventricular contraction, it is not an accurate measurement of this relation. However, it is quite probable that a change in the P-R interval reflects with reasonable accuracy a comparable change in the time relation of auricular and ventricular contraction. It cannot be the P-R interval per se but the time relation of dynamic factors, the As-Vs interval, which influences the first sound. If there is present asynchronism in either auricular or ventricular contraction, there will be differences in As-Vs intervals on the two sides, depending in extent on the degree of asynchronism. Thus, if the P-R interval in any beat has associated with it different As-Vs intervals on the two sides of the heart, the effect of auricular contraction on the two ventricles will not be strictly comparable. For example, if one ventricle begins to contract at the summit of the wave of auricular filling, it is likely that the other ventricle will begin to contract on the ascending or descending slope of the wave, or possibly even before or after the wave.

If it were possible to obtain accurate measurements of As-Vs intervals on the two sides in patients, the problem would be greatly simplified. It occurred to us that the intervals between the beginnings of P waves and sound components might serve, at least to a certain extent, as indicators of the As-Vs intervals on the two sides

of the heart. Such measurements fail to reflect asynchronism in the waves of ventricular filling due to auricular contraction. However, if, as stated by Wiggers (8), main sound vibrations are associated with the rise of intraventricular pressure during the isometric contraction phase, it may reasonably be assumed that the interval between the two components should reflect with considerable accuracy asynchronism in the onset of the isometric contraction phase of the two ventricles. On the basis of these considerations the variations in the sound components were studied with respect to the time relation of each component to the preceding P wave.

The relation in Case I between the variations in amplitude of the two sound components and the interval from the beginning of the P wave to each sound component are shown in Table I. The table shows that there is actually nothing haphazard about the variations of the sound components, even though they do vary independently of each other. Each has its own clear cut definite relationship to the P wave. Furthermore, it is of interest to note that the time zone of intervals between P waves and sound components, during which waves of large amplitude and waves of small amplitude are recorded, differ but little for the two components. These findings are, therefore, in accord with the postulate that one component is produced in one ventricle and the other component in the other ventricle, and that the apparently independent variations are actually dependent on different As-Vs intervals on the two sides.

The objection may be raised that variations in major vibrations from beat to beat, even in a continuous strip of tracing, do not necessarily indicate variations in loudness of sounds, although intensity is supposed to vary as the square of amplitude times frequency. As stated above, we have paid considerable attention to this point in the study of first sounds and have found that the loudest sounds, as judged by auscultation, are recorded with major vibrations of widest amplitude. However, this correlation is of no particular importance so far as our present analysis is concerned. The significance of the variations in amplitude of vibrations, considered as evidence as to whether both components of sound origi-

TABLE I

The influence of various A-V time relations on the two components of split first heart sounds. In each case the data shown are obtained from a single continuous tracing.

Time interval			Maximum amplitude of sound vibrations	
P-R or R-P	P to 1st sound component	P to 2nd sound component	1st component	2nd component
seconds	seconds	seconds	mm.	mm.

## Case I

R-P				
0.11	-0.06*	0.04	8	7
0.09	-0.04	0.06	10	9
0.07	-0.02	0.08	9	13
P buried in QRS			8	42
P-R				
0.02	0.07	0.17	8	42
0.03	0.08	0.18	9	48
0.05	0.10†	0.20	18	48
0.07	0.12	0.22	39	38
0.10	0.15	0.25	34	24
0.11	0.16	0.26	21	22
0.12	0.17	0.27	25	20
0.16	0.21	0.31	21	5
0.18 to 0.80	0.23 to 0.85	0.33 to 0.96	8 to 11	5 to 8

## Case II

P buried in QRS			9	7
			9	15
			11	14
			12	8
			13	12
P-R				
0.01	0.05	0.13	15	18
0.02	0.06	0.14	14	20
0.05	0.09	0.17	13	12
0.06	0.10	0.18	22	10
0.08	0.12	0.20	53	16
0.09	0.13	0.21	51	15
0.11	0.15	0.23	42	11
0.14	0.18	0.26	16	7
0.15	0.19	0.27	30	12
0.18 to 0.55	0.22 to 0.59	0.30 to 0.67	6 to 14	6 to 13

\* The minus signs indicate that the P wave followed rather than preceded the sound component.

† The numbers in heavy type indicate the time relations which are associated with significantly increased amplitude of sound vibrations.

nated in a single ventricle or one from each ventricle, remains the same whether the variations in amplitude reflect variations of intensity or some other quality of the sounds.

**Case II.** A young woman, 31 years old, had complete A-V heart block, slightly increased intraventricular conduction time (duration of QRS complex 0.11 second), a widely split first heart sound and a variation in loudness of the first sound from beat to beat. In these respects the findings were similar to those of Case I, except that the duration of QRS was less. Numerous records of heart sounds were made, a sample of which is shown in Figure 4. In Figure 5, representative beats were selected from a continuous tracing to show the effect of various A-V relations on the two components of the first sound. The measurements of amplitude of the largest vibration recorded for each sound component in a continuous strip of tracing are recorded in Table I, under Case II. The data are arranged with reference to the duration of the intervals between P waves and sound components, as in Case I. The variations in amplitude of vibrations of the first component were considerably greater than those of the second component, whereas in Case I the variations were slightly greater in the second component. However, the data in Case II (Table I) make it clear that in beats with relatively short As-Vs intervals the two components vary independently of each other. The zone of As-Vs intervals with maximum amplitudes of the first component is clearly displayed. Although the shortest As-Vs time intervals associated with maximal vibrations of the second component could not be clearly determined because the P wave was buried in the QRS complex, the longest intervals associated with maximal vibrations were nearly the same as in the case of the first component. The number of observations that could be made in a single strip, such as the one used, may be regarded as too few to be convincing. Similar data, however, were also obtained from other strips of tracing made at various times.

The variations in the amplitude of the major vibrations of the two components of the first sound with reference to their relations to P waves in this case are of the same nature as those observed in Case I. It is, therefore, unnecessary to repeat the points made to justify the conclusion that one component originated in the left ventricle and the other in the right ventricle.

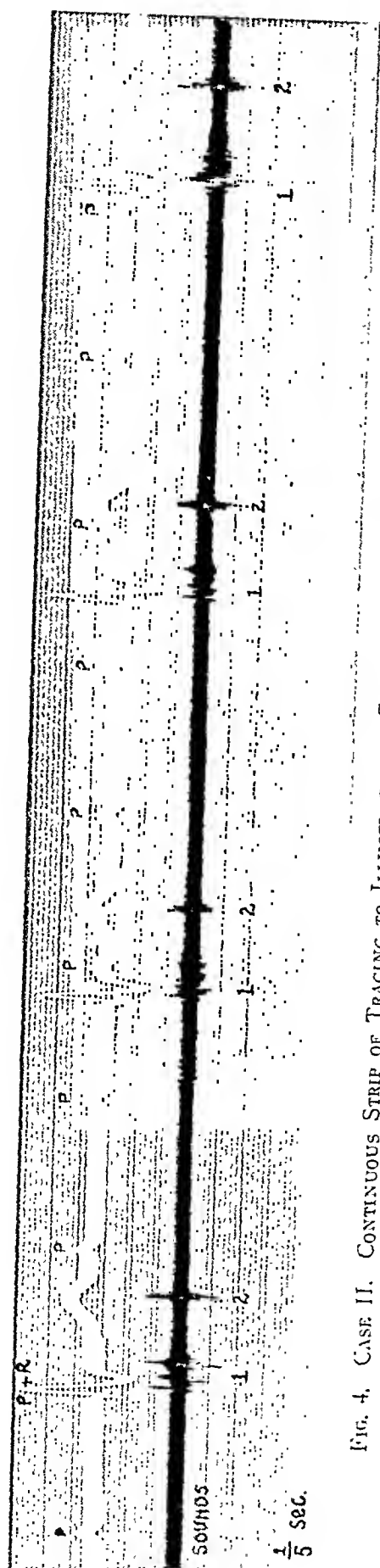


FIG. 4. CASE II. CONTINUOUS STRIP OF TRACING TO ILLUSTRATE THE CHANGES IN THE FIRST SOUND IN SUCCESSIVE BEATS.

*Case III.* A man, 65 years old, had ventricular escape. Each cycle of the abnormal mechanism was terminated by an apparently premature ventricular beat. The apparently premature ventricular beat occurred whenever an auricular beat lagged far enough after the preceding ventricular beat to permit A-V conduction. The QRS complexes had a duration of 0.07 second, indicating the absence of intraventricular conduction defect. The first heart sound was found on auscultation to be split and also to vary markedly in loudness from beat to beat.

The sound tracing (Figure 6) shows that variations in amplitude of major vibrations of the two components of the first sound were, to a certain extent at least, independent of each other. Thus the amplitude might be (1) large in both components, (2) small in both, (3) large in the first and small in the second or (4) small in the first and large in the second. These various combinations are similar to those found in Cases I and II. The tracing shows that marked variations in amplitude of vibrations are associated with changes in P-R time intervals. No accurate analysis of this relation, however, can be made in Case III because of the fact that the ventricular arrhythmia also influences the sounds (10). Nevertheless, the fact that the two components of sound are capable of varying independently of each other points, as in Cases I and II, to the postulate that one component originates in the left ventricle and the other component in the right ventricle.

The fact that in Case III there is no delay in intraventricular conduction, as in Cases I and II, to account for asynchronism in the early phases of ventricular contraction on the two sides, requires comment. In our previous study it was found that asynchronism in the beginning of ejection from the two ventricles occurred in cases with normal intraventricular conduction and split first sounds (6). Whether these differences in the ending of the isometric contraction phase (ejection) were due to inequality in its duration on the two sides or to asynchronism in onset or to both, could not be determined. The differences in the two components of sound depending on A-S-Vs intervals could be equally well accounted for on theoretical grounds by asynchronism in auricular systole on the two sides. The particular point we

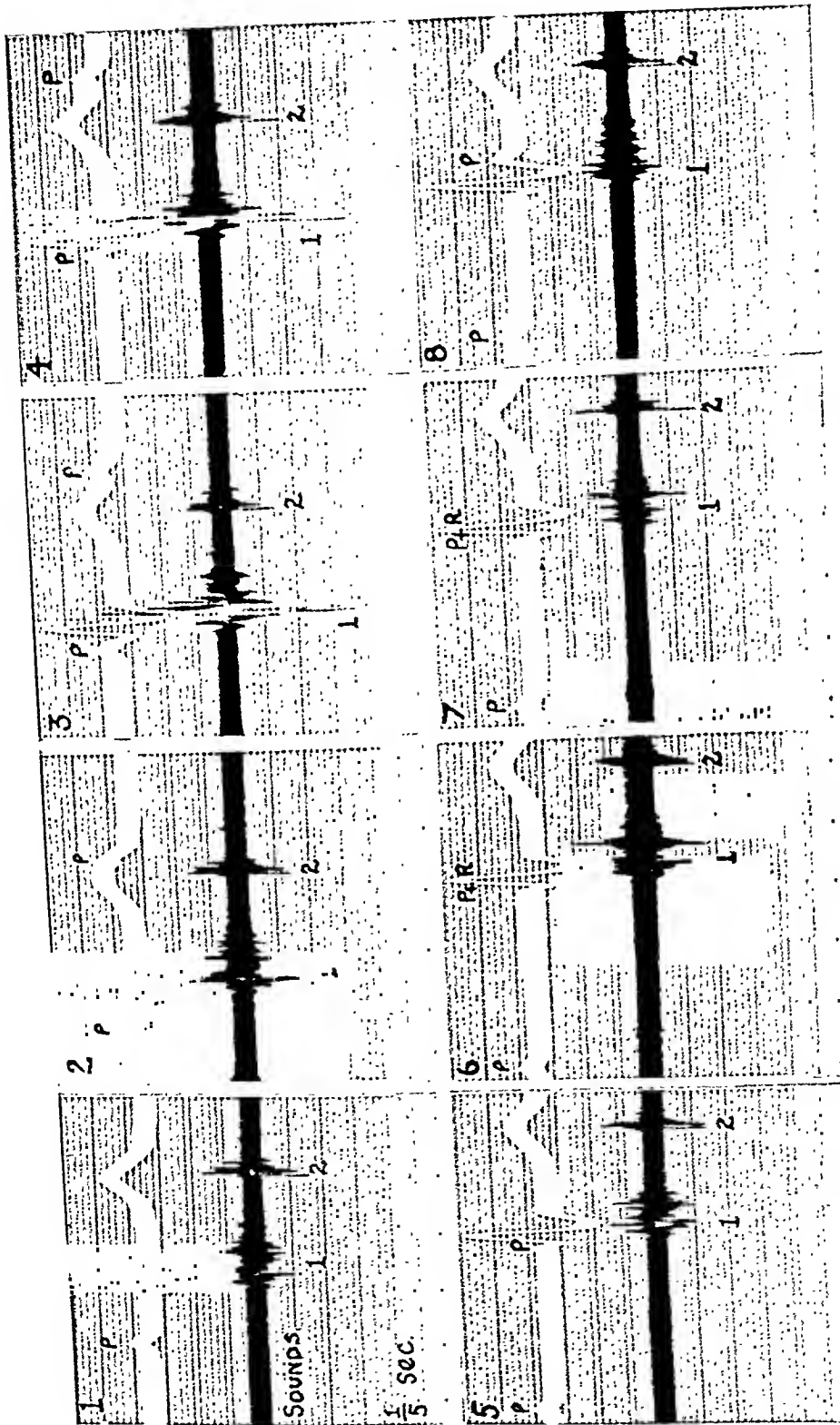


FIG. 5. CASE 11. BEATS SELECTED FROM A CONTINUOUS STRIP OF TRACING TO SHOW THE EFFECT OF VARIOUS P-R TIME RELATIONSHIPS ON THE RECORDED AMPLITUDE OF VIBRATIONS OF THE FIRST HEART SOUNDS.

As the P-R interval shortens there is noted first increase in amplitude of vibrations of the first component and, when the interval becomes very short, the vibrations of the second component are increased. When the P wave is wholly buried in the QRS complex (7th beat) only the second component is influenced. In this case the extent of the variations in the second component are not nearly so great as those of the first component. The vibrations of a soft systolic murmur are noted following the second component.

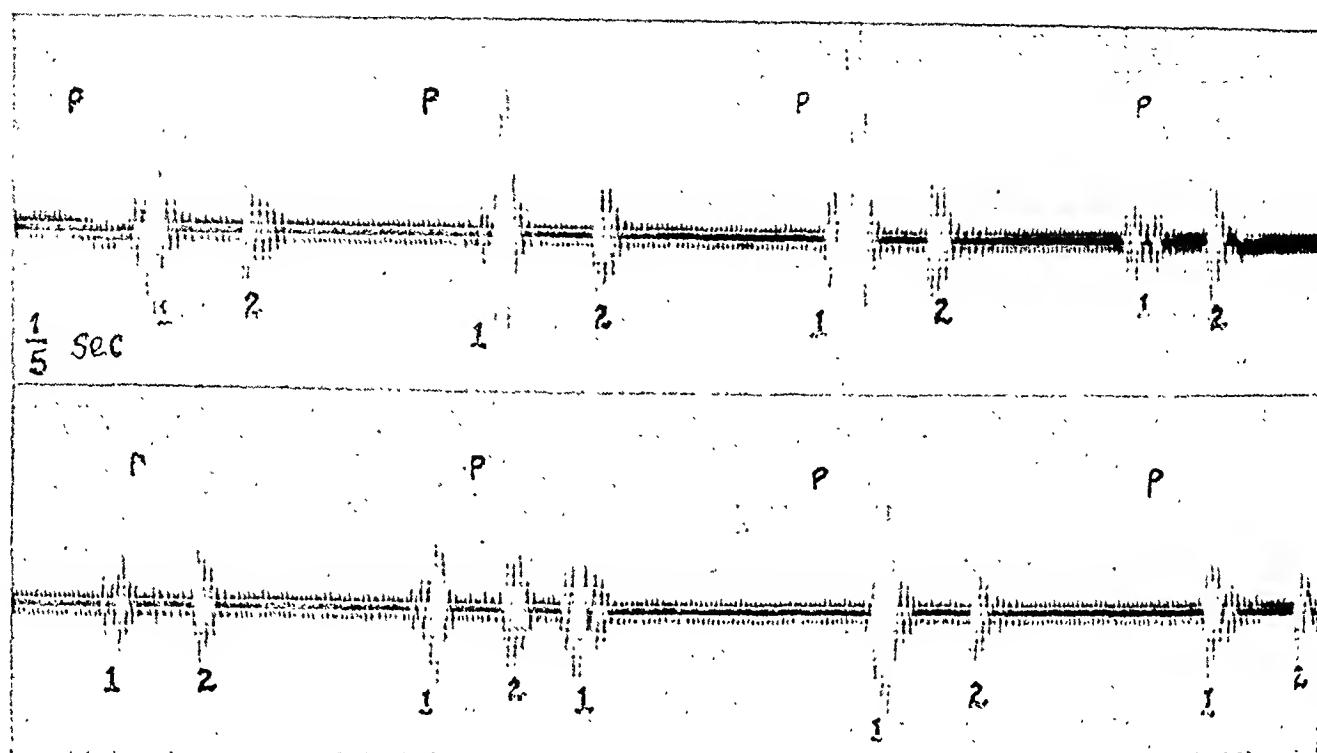


FIG. 6. CASE III. THE LOWER STRIP IS A CONTINUATION OF THE UPPER

The variations in the two components of the first sound, particularly those in the lower strip, are not entirely due to changes in A-V time relation but are doubtless also influenced by the ventricular arrhythmia.

wish to emphasize in this connection is that, although the mechanism responsible for the variations in sound components cannot be demonstrated completely by the studies thus far made, the occurrence of independent variations depending on changes of As-Vs intervals in cases without delay in the spread of intraventricular conduction is in no way inconsistent with the postulate that one component is contributed by each ventricle.

*Case IV.* A man, 55 years old, had complete heart block but no intraventricular conduction defect (duration of QRS complex 0.08 second). Auscultation revealed the fact that the first heart sound was prolonged but not definitely split and that there were variations in loudness of the sounds, certain beats being much louder than others. A representative strip of the sound tracing obtained in this case is shown in Figure 7. Marked variations in amplitude of major vibrations of the first sound are present, depending on the duration of the As-Vs interval. In this respect the behavior of the first sound corresponds with that observed in most other cases of complete heart block.

The sound tracing (Figure 7) shows, during a certain range of P-R relations in which large

vibrations occur, that as the interval shortens, the position of the largest vibrations shifts from the first part of the prolonged sound to the last part. This behavior is so similar to that observed in the two components of the split first sound in Cases I and II as to suggest that prolongation of the sound is due to slight asynchronism of the two components, not sufficient to cause actual separation from each other, and that the mechanism responsible for the variations is essentially the same as in Cases I and II.

#### COMMENT

All the facts available at this time appear to be consistent with the postulate that in split first heart sounds one major component is contributed by the right ventricle and the other major component by the left ventricle. The alternative view, that both components arise in the left ventricle or that both receive contributions from each ventricle, seems improbable, at least so far as the cases we have studied are concerned. Furthermore, there is some evidence that single first sounds are often contributed to by a component from each ventricle, better synchronized than in the case of the split sounds. It is, of course, probable that in certain

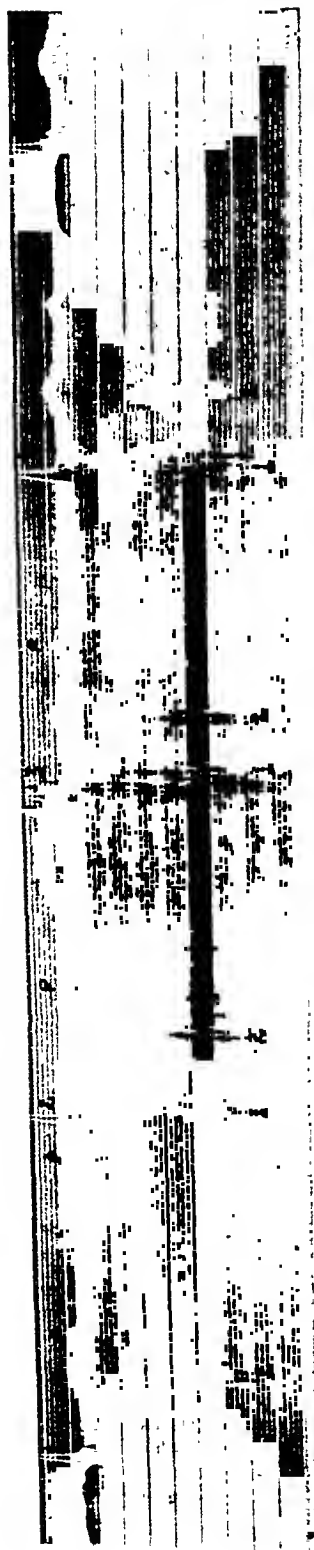


FIG. 7. CASE IV. AS THE P-R INTERVAL SHORTENS THE FIRST CHANGE IN THE PROLONGED FIRST SOUND IS INCREASE IN AMPLITUDE OF THE EARLY VIBRATIONS.

(Compare the first and second beats with the fifth.) In association with shorter P-R intervals vibrations are large throughout the sound (third beat). With an extremely short P-R interval the late vibrations are larger than the early vibrations (fourth beat).

cases, possibly in many cases, the contribution from one ventricle may be negligible.

It is of interest that the duality of the first sound appears to be analogous in certain respects to that of the second sound. We have previously shown that (1) splitting of the second sound is associated with asynchronism in the ending of the ejection period from the two ventricles and must be due, therefore, as has been generally believed, to asynchronism in closure of the aortic and pulmonic valves (6); (2) each component is a short sharp sound; and (3) imperfect synchronization results in a prolonged second sound (13). It is, therefore, highly probable that in most cases with single second sounds both sides contribute a component to the sound. Furthermore, it is noteworthy that bundle branch block, with its marked asynchronism of the entire ejection phase, tends to be associated with rather wide splitting of both the first and second heart sound (6).

Our studies do not solve the problem as to how a sound component is produced in a ventricle, although they do show rather clearly that both sound components may be modified tremendously by relatively slight changes in A-S-Vs intervals. Furthermore, they indicate that in studies of the first sound the nature of the duality must be borne in mind, particularly in any investigation of the time relations of the sound. Failure to do so may result in serious error.

In view of the remarkable relation between the duration of A-S-Vs intervals and the loudness of the first sound, which is so clearly shown in dealing with single sound components, the recent contribution of Dock (14), regarding the mechanism of production of the first sound, requires comment. Dock has performed experiments on the A-V valves which he believes support the view that the first sound is produced by vibration of these valves. The experiments, however, which brought about modification or disappearance of the first sound included drawing a ligature tightly around the A-V groove, which apparently trapped blood within the ventricles but did not prevent them from "contracting vigorously." Thus, so-called isometric contraction with a volume of blood within the ventricles was obtained, the A-V valves being held presumably immobile by the ligature around the A-V groove.

In our opinion it is difficult to draw conclusions regarding the first sound from this interesting experiment. The production of sound requires the application of an appropriate force to a structure capable of vibrating within the frequency range of audibility. Therefore, if conclusions are to be drawn from experiments in which one source of vibrations is eliminated, it is necessary to control the application of force so that possible vibrations of other structures are not suppressed. Wiggers has pointed out that variations in systolic discharge and arterial resistance markedly influence production of sound (15). However, the fact illustrated in Table I of this paper, that a change in the P-R interval of not more than 0.03 to 0.04 second is associated with change from large to small amplitude of sound vibrations without material change in the pulse volume, as shown by optically recorded pulse tracings, indicates clearly and significantly the delicate balance of factors concerned in production of heart sounds.

There are a number of facts which support the view that the factor initiating the major vibrations of the first sound is the rise of intraventricular pressure, which results from the force exerted by ventricular contraction on the ventricular content, during the isometric contraction phase. So far as we are aware, there is no evidence against such a postulate. Observations on patients with various levels of blood pressure suggest that the gradient of this rise of intraventricular pressure may be quite as important in production of sound as its extent, possibly even more so. Thus, some patients with low systolic blood pressure have louder first sounds than other patients with high systolic pressure. It seems quite possible that experiments of a type which presumably modify ventricular dynamics so much as is to be expected from ligation of the auriculoventricular groove, might modify to a considerable extent the gradient and the extent of the rise of intraventricular pressure. In view of the fact that, as has been demonstrated by the study of A-S-V intervals, even slight changes may cause tremendous variations in sounds, it does not seem unreasonable to suppose that the disappearance of sounds, noted by Dock in the course of his experiment, may be

due at least in part to changes in ventricular dynamics rather than wholly to immobilization of the A-V valves. Therefore, it does not appear to be established that immobilization of the valves *per se* is the important factor in the suppression of the sounds. The question as to what anatomical structure or structures supply the most important vibrations during ventricular contraction remains, in our opinion, an unsolved problem.

We have provisionally adopted the following views regarding the first heart sound, subject to modification as a result of future investigation: (a) the important duality of the first sound is due to the contribution of both right and left ventricular components; (b) each component is produced during the ventricular isometric contraction phase and is associated with the period of rapidly rising pressure; (c) factors which modify the curve of rising pressure will modify the sound. One of the most important of such factors is the wave of ventricular filling produced by auricular contraction, which, by influencing (1) intraventricular volume, (2) initial intraventricular pressure and possibly also (3) the position of the A-V valves at the instant ventricular contraction begins, may exert a profound influence on sound production. Clinical observation indicates that the physiological state of the ventricles is also a factor. Thus, in hyperthyroidism the first sound tends to be loud; when the ventricular action is weakened, as in the common type of bundle branch block, acute coronary occlusion or a moribund state, the first sound tends to be faint. However, weakened ventricles, unable to maintain cardiac compensation, are frequently capable of contributing loud sounds. (d) The time relations of the isometric contraction phase in the two ventricles determines whether the first sound will be short, prolonged or split. It is probable, however, that certain first sounds are short because of failure of one ventricle to make a significant contribution to the sound. (e) Heart sounds are influenced by the physical properties of the structure or structures set into vibration and are modified by the physical properties of structure through which these vibrations are transmitted to the surface of the chest. It has not been determined what structure or structures contribute the most important sound vibrations.



## SUMMARY

1. Two cases are presented, both exhibiting complete heart block and split first heart sounds. Heart sound tracings showed marked variations in amplitude of vibrations in each component of the split sounds. During certain ranges of P-R relations the variations in the two components appeared to be independent of each other.

2. Evidence previously obtained suggests that in cases with split first heart sounds there is asynchronism in the isometric contraction phase of the two ventricles, corresponding to the split between the two components. This affords a reasonable explanation for the variations in the two sound components, namely, that they are due to different As-Vs intervals on the two sides of the heart. Furthermore, the range of As-Vs intervals associated with increased amplitude of waves differs but little for the two components. Thus, the apparently haphazard variations in the second component are found to be of the same nature as the variations in the first component. Both components behave as do single first sounds when they are influenced by As-Vs intervals which change from beat to beat.

3. In a number of beats recorded, particularly in Case II, the P wave falls so late with reference to the first sound component that auricular contraction could not have materially influenced this component, although marked increase of amplitude in the waves of the second component occur.

4. In Case III (in which changes in As-Vs intervals are due to ventricular escape) independent variations in the two components of the first sound occur, which are similar to those observed in Cases I and II. This case is not as favorable for analysis as Cases I and II because the presence of ventricular arrhythmia may also influence the first heart sound.

5. Case IV shows complete heart block and a prolonged first sound. During a certain range of P-R relations the first part of the sound is represented by vibrations with large amplitude, and during a slightly shorter range the last part of the sound is represented by sounds with large amplitude. Since this behavior corresponds to that of the two components of a split first sound during certain ranges of P-R intervals, it sug-

gests that the prolongation of the first sound is due to slight asynchronism of the two major sound components.

6. All the findings in our cases are in accord with the postulate that one main component of the first sound is contributed by one ventricle and the other main component by the other ventricle. They lend no support to the view that both components arise in a single ventricle and, as a matter of fact, cannot be accounted for on such a basis.

7. It is suggested, on the basis of present knowledge, that major sound vibrations in each ventricle are associated with the period of rapidly rising pressure of that ventricle during the isometric contraction phase and that factors which modify the curve of rising pressure also modify production of sound. The volume of sound does not necessarily parallel the extent of rise of intraventricular pressure; the sounds are capable of varying independently of the output (as shown by optically recorded arterial pulse waves); it is probable that the gradient of rise of pressure is the most important factor. It has thus far not been determined what structure or structures contribute the main sound vibrations in response to the application of force initiated by the contraction of the ventricular muscle upon the ventricular contents.

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# COMPARATIVE CALORIGENIC ACTION OF NORMAL AND PATHOLOGICAL THYROID GLANDS ADMINISTERED IN EQUI-THYROXINE DOSES

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The purpose of this communication is to present evidence concerning two important questions of thyroid physiology.

1. Is the calorogenic activity of the thyroid gland proportional to its thyroxine content as determined chemically or to its total iodine?

2. Is the toxicity observed in Graves' disease caused entirely by an excessive secretion of normal thyroid hormone or is it due wholly or in part to the elaboration of an abnormal substance more toxic than the normal hormone?

In reference to the first question, the rôle of thyroxine in relation to the activity of the thyroid gland, there is disagreement among the several workers in the field. Hunt and Seidell (1), using a method in which the minimum lethal dose of acetonitrile for thyroid treated mice is determined and compared with that for untreated control mice, found a constant parallelism between the activity and the total iodine content of the thyroid preparations tested. Krogh and Lindberg (2) found that the increase in oxygen consumption of guinea pigs was proportional to the total iodine of dried thyroid gland when they used normal hog thyroids and the thyroid glands of patients with simple goiter or with exophthalmic goiter successfully treated with iodine. When, however, glands from exophthalmic goiter patients who were not treated with iodine or who failed to respond to treatment were used, the biological activity per milligram of total iodine was less than that of normal glands. Means, Lerman and Salter (3), using preparations analyzed for thyroxine by the method of Harington and Randall (4), have concluded from work with myxedema patients that the activity of desiccated thyroid gland is proportional to its total iodine content. Working also with myxedema patients, Thompson, McClellan, Thompson and Dickie (5) reported similar results in their earlier work but more recently (6) have obtained figures that lead them to doubt their first

conclusions and to consider that the question remains unanswered.

Mörch (7) studied six commercial thyroid preparations by measuring the carbon dioxide output of mice before and after dosage but failed to find a parallelism between the total iodine content and the effect on metabolism. The same conclusion was reached by Kreitmair (8) who followed the loss in weight of guinea pigs after the administration of the glands.

Gaddum and Hetherington (9), using Mörch's method, found only a rough proportionality between the calorogenic activity of a series of thyroid gland preparations and their thyroxine content, estimated according to the method of Harington and Randall. On the other hand, Rotter and Mecz (10) and also Sjögren and Lundgren (11) obtained a fairly uniform proportionality between thyroxine content and potency as measured by the loss of weight in guinea pigs by the method of Freud and Nobel (12) and Kreitmair (8) obtained a fairly uniform proportionality between the calorogenic effect and the thyroxine content.

To obtain evidence with reference to these questions it was decided to make comparisons between the calorogenic activity of a commercial thyroid preparation, used as a standard, and desiccated thyroid preparations in which the ratios of thyroxine iodine to total iodine varied widely from the standard. With such a series of preparations it should be apparent whether the activity is parallel to the total iodine or to the thyroxine iodine content. It has been shown previously in this laboratory that pathological glands may be found with widely varying ratios of thyroxine iodine to total iodine.

## METHOD

The preparations studied were: (1) a commercial thyroid powder of Burroughs Wellcome

Co. (the "standard"), (2) nine human thyroids (both normal and pathological), (3) another commercial preparation, (4) a specimen of hog thyroglobulin, and (5) pure crystalline thyroxine. The thyroid preparations were analyzed for total iodine by a modification of Kendall's method (13) and for thyroxine by the method of Leland and Foster (14). It is believed for reasons previously given (14) that the figures obtained by the method of Leland and Foster more closely approach the true thyroxine values than do those by the method of Harington and Randall (4) which gives much higher results.

Since it was considered advisable to study individual human thyroid glands the available material for experimentation was limited and it became necessary, therefore, to use small animals. The method devised by Mørch in which the test animals used are mice was attempted but in our hands (15) proved unsatisfactory. A change was made, therefore, to the procedure recommended by Krogh and Lindberg (2), that of determining the oxygen consumption of guinea pigs. In the main the experimental details suggested by these workers were adhered to closely. Minor changes made in the method will be described together with details of diet not mentioned by Krogh and Lindberg.

Male guinea pigs, from 600 to 800 grams in weight, obtained from the same stock throughout the investigation, were fed a dry diet consisting of two parts by weight of crushed oats to one part of bran supplemented by carrots and 80 grams of lettuce daily. The animals were transferred to starvation cages from 12 to 14 hours before the metabolism was determined. In spite of the long daily starvation period, the animals maintained their weight or gained on the diet except in two or three instances. In those cases where a steady weight loss was noted during the preliminary period the animals were discarded.

After an interval of several days during which the animals became adjusted to the experimental conditions, the normal resting metabolism (consumption of oxygen) was determined daily, including Sunday, over a period of six to ten days before thyroid administration was begun. Since some animals showed considerable variation in their  $O_2$  consumption from day to day, this relatively long period was considered necessary to obtain a fair average normal. A mean for each animal was calculated from all of the values found over this period. The average deviation of the individual values from their mean was 3.4 per cent.

Immediately following the determination of the normal, thyroid administration was begun and was continued over a fourteen day period. Every other day the animal was given by mouth a dose of a watery suspension of gland material. No difficulty was encountered in giving the dose as preliminary training in drinking from a pipette had been accomplished during the period of adjustment. Throughout the experimental period the metabolism was determined daily. A 150 cc. spirometer was used and the oxygen consumption recorded on Benedict-Roth kymograph sheets of the type used with their portable apparatus. These sheets also served as a record of the activity of the animal, for any movement within the metabolism chamber changed the pressure and caused a rise and fall of the needle on the graph. Instead of using a pump, the respiration chamber was aerated by means of a rocking device (16) containing 40 per cent potassium hydroxide for the absorption of carbon dioxide. Analysis of the air in the system showed that the carbon dioxide was promptly absorbed. The determinations themselves covered a period of one hour instead of 30 minutes. The first 15 minute period during which the temperature throughout the system was becoming constant was discarded. Three ten minute periods were then obtained; if all three or two of these three periods agreed within 5 per cent, the results were accepted and averaged.

All results are expressed in oxygen consumption per square meter of surface area per minute using Meeh's formula for surface area,  $S = k\sqrt[3]{w^2}$  ( $w$  = weight in kgm.), with Rubner's factor,  $k = .085$ , for guinea pigs. The average normal resting metabolism of 116 male guinea pigs was found to be 97 cc. oxygen per square meter per minute with a dispersion of  $\pm 13$  and a standard deviation ( $\sigma = \sqrt{\sum x^2/N}$ ) of 5.3. The percentage increase in oxygen consumption following administration of thyroid was computed on the basis of the individual animal's normal average and not on the group normal average as was done by Krogh and Lindberg.

#### EXPERIMENTAL

In order to establish the relation between size of dose and magnitude of response, the standard preparation, Burroughs Wellcome, was given in amounts containing 4.50, 2.25, 1.69, 1.13 and 0.56 mgm. of total iodine per square meter of surface. The corresponding thyroxine iodine contents of the standard at these levels of dosage were 1.05, 0.52, 0.39, 0.26, and 0.13 mgm. In Figure 1 a complete picture is given of the increases in oxygen consumption observed over the entire 14 day period at each of the different levels of dosage. The curves are composite curves obtained by averaging each day the oxygen absorption of all of the individual animals of the group receiving the dose. From the lowest curve (0.13

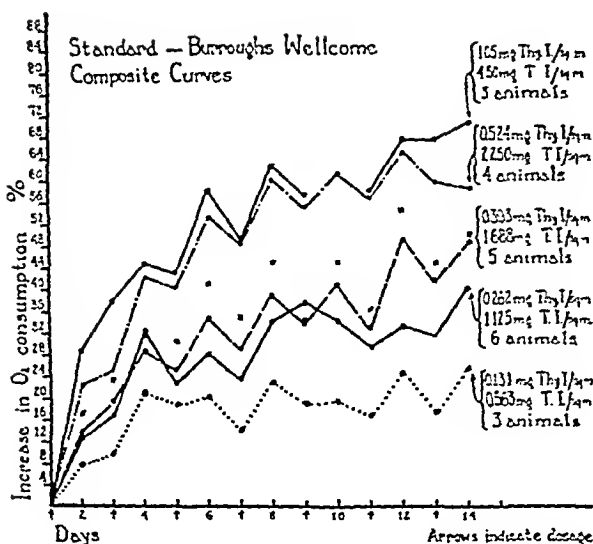


FIG. 1. COMPOSITE CURVES OBTAINED BY AVERAGING DAILY THE OXYGEN CONSUMPTION OF ALL OF THE ANIMALS OF EACH GROUP RECEIVING THE STANDARD PREPARATION, BURROUGHS WELLCOME COMPANY THYROID.

Gland was administered every other day for 14 days at five different levels of dosage. Crosses indicate values obtained in Curve 3 when two unsatisfactory animals were omitted from the group.

mgm. thyroxine iodine) to the next above (0.26 mgm. thyroxine iodine) a definite increase in oxygen absorption is observed over the entire period. The third curve (0.39 mgm. thyroxine iodine) is not significantly higher than the second curve (0.26 mgm. thyroxine iodine) until the end of the dosage period is reached. This is due to the inclusion in the series of the low results obtained on two animals that could not be kept awake until toward the end of the experiment. The crosses indicate the position of the curve when these animals were excluded from the series. The last three days of the experimental period were not complicated by this factor. Although the dose of thyroxine iodine given to the animals of the fifth curve (1.05 mgm. thyroxine iodine) was twice that of the fourth curve (0.52 mgm. thyroxine iodine) the oxygen consumption was only a little greater showing that the amount given was outside the range of sensitivity. At this high level of dosage the increase in oxygen consumption is no longer proportional to the dose administered. The total iodine content of the dose at this level was 4.5 mgm. per square meter of surface, the

amount chosen by Krogh and Lindberg as their standard dose.

Following the procedure of Krogh and Lindberg, a value was obtained by averaging the increases in oxygen consumption observed on the twelfth, thirteenth, and fourteenth days of the experimental period which was regarded as the full effect of the dose administered. When the standard preparation was given in amounts containing 2.25, 1.69, 1.13 and 0.56 mgm. of total iodine per square meter of surface, a linear relationship between the increases in oxygen consumption is observed as may be seen by referring to Figure 2. The mean increases in oxygen consumption obtained at the four points which locate the line are respectively 61, 47, 35 and 22 per cent. The amount of thyroxine iodine given at each of these levels was 0.52, 0.39, 0.26 and 0.13 mgm. per square meter. Within this range of dosage, therefore, a difference of 0.13 mgm. thyroxine iodine given results in an appreciable difference in oxygen consumption. Beyond the highest point shown in Figure 2 the curve flattens

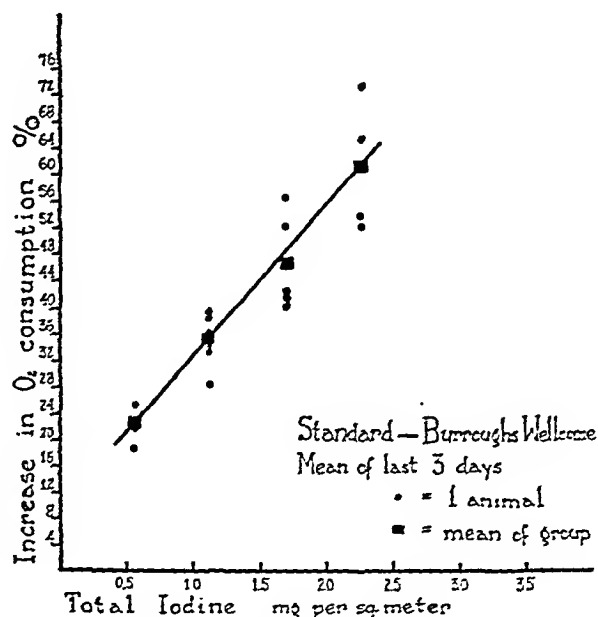


FIG. 2. CURVE SHOWING THE INCREASE IN OXYGEN CONSUMPTION OF GUINEA PIGS RECEIVING THE STANDARD PREPARATION, BURROUGHS WELLCOME COMPANY THYROID, AT FOUR DIFFERENT LEVELS OF DOSAGE.

Each solid dot represents the mean oxygen consumption on the 12th, 13th, and 14th days of the dosage period.

out, since an increase of only 68 per cent results when 1.05 mgm. thyroxine iodine (4.5 mgm. total iodine) per square meter is administered. Krogh and Lindberg used 4.5 mgm. of total iodine as their standard dose but obtained an increase in oxygen consumption of only 30 per cent as compared with our increase of 68 per cent at that level. The difference in activity between our standard preparation and the one employed by Krogh and Lindberg is probably explained by a difference in thyroxine content. Since toxic symptoms were observed with thyroid doses producing increases in oxygen consumption of 61 and 47 per cent it was decided to use, when sufficient gland was available, the 35 per cent level of dosage with which to make comparisons of preparations to be studied.

The accompanying weight losses observed with the various dosages of the standard preparation are shown in Table I. As there is only a rough correlation between weight loss and thyroid dosage, the determination of the oxygen consumption is the more delicate method of estimating biological activity.

Having established the mean increase in oxy-

TABLE I  
*Weight losses accompanying different levels of dosage of Burroughs Wellcome thyroid*

Dosage per square meter		Average increase in oxygen consumption	Average weight loss on 14th day
Total iodine	Thyroxine iodine		
mgm.	mgm.	per cent	per cent of average initial body weight
0.56	0.13	22	6.6
1.13	0.26	35	6.6
1.69	0.39	47	12.8
2.25	0.52	61	13.3
4.50	1.05	68	22.3

gen consumption produced by administration of standard normal thyroid in dosages indicated above, the next step was to compare the calorigenic effect of the standard with that of other thyroid preparations in equi-thyroxine iodine dosage. Twelve preparations were compared in equi-thyroxine doses with the standard. These consisted of nine human thyroid glands (both normal and pathological), a commercial thyroid preparation, hog thyroglobulin and pure crystalline thyroxine. In Table II may be found the chemical analyses and types of glands employed. All anal-

TABLE II  
*Iodine partition of thyroid gland preparations*

Preparation	Description	Iodine content dry gland			Dose administered*	
		Total	Thyroxine	Thyroxine I Total I	Thyroxine iodine	Total iodine
		per cent	per cent	per cent	mgm. per square meter	mgm. per square meter
Burroughs Wellcome Co. Standard.....	Commercial preparation	0.254	0.0593	23.3	0.13 0.26 0.39 0.52 1.05	0.56 1.13 1.69 2.25 4.50
Swan-Meyer.....	Commercial preparation 14425, Number 219	0.082	0.011	13.4	0.13	0.98
Case 9.....	Human: Normal thyroid. Series Gutman et al.	0.629	0.0687	10.9	0.26	2.30
Case 11.....	Human: Normal thyroid. Series Leland and Foster	0.237	0.0742	31.3	0.26	0.82
Case P.....	Human: Non-toxic nodular goiter	0.049	0.0041	8.4	0.13	1.57
Case R.....	Human: Non-toxic nodular goiter	0.0575	0.0041	7.1	0.13	1.84
Case H.....	Human: Toxic diffuse goiter†	0.143	0.0077	5.4	0.13	2.44
Case B.C.....	Human: Toxic diffuse goiter†	0.215	0.0189	8.8	0.26	3.00
Case M.....	Human: Toxic diffuse goiter†	0.360	0.109	30.3	0.26	0.87
Case W.....	Human: Toxic diffuse goiter†	0.237	0.0622	26.2	0.26	1.00
Case M.C.....	Human: Toxic diffuse goiter†	0.198	0.028	14.1	0.13	0.93
Thyroglobulin.....	Hog thyroids	0.622	0.213	34.2	0.26	0.77

\* Dose administered every other day for 14 days.

† Patient received iodine before operation.

yses for total iodine and thyroxine iodine were made by Dr. G. L. Foster to whom we are greatly indebted. As the thyroxine per cent of total iodine is low the best glands for comparison with the standard are Cases 9, P, R, H and B.C. The total iodine contents of these preparations are respectively 2, 2.8, 3.3, 4.3 and 2.7 times as great as in an equi-thyroxine dose of the standard.

# RESULTS

The experimental results obtained over the entire fourteen day period of dosage are shown in Figures 3 to 14, and the results found by averaging the oxygen consumption on the 12th, 13th and 14th days are given in Table III.

Figures 3 to 14 show composite curves obtained by averaging the animals of each group. In each figure the preparation to be compared with the standard is shown together with two Burroughs Wellcome curves, one the exact thyroxine iodine equivalent and the other the nearest total iodine equivalent taken from Figure 2. In the cases where none of the standard curves represented an approximate total iodine equivalent, the curves above and below the theoretical are shown. In these curves the increase in oxygen consumption obtained with the thyroid preparation investigated would approximate that obtained with an equi-thyroxine iodine dosage of the standard if the calorigenic effect were proportional to the

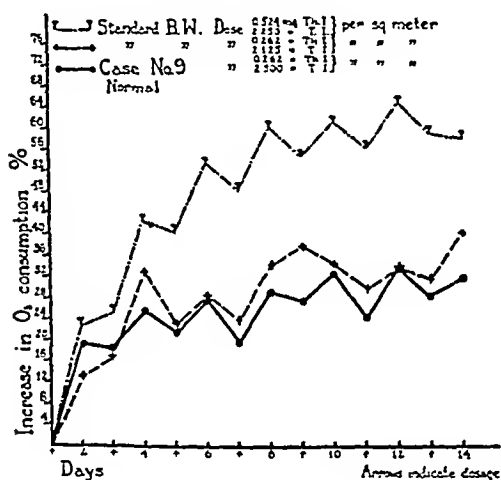


FIG. 3.

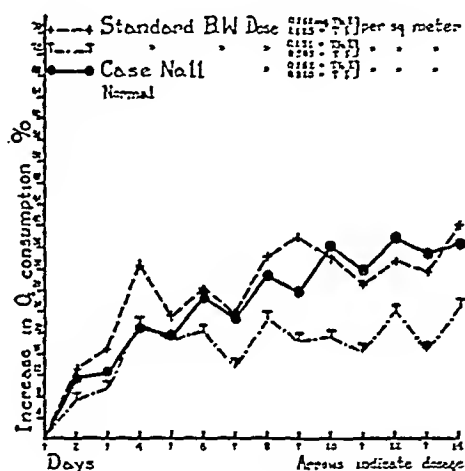


FIG. 4.

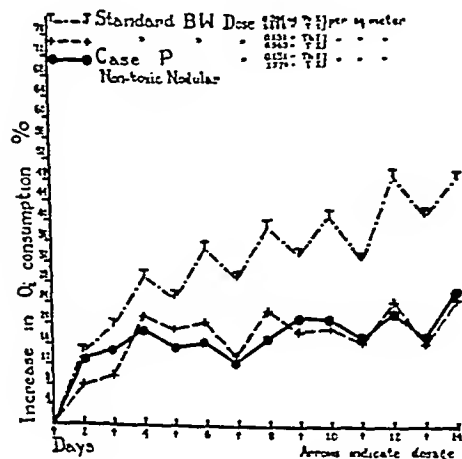


FIG. 5.

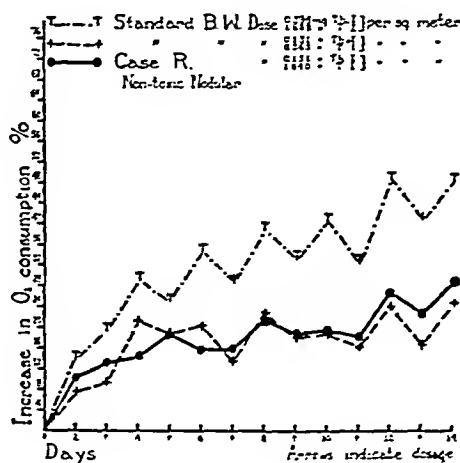


FIG. 6.

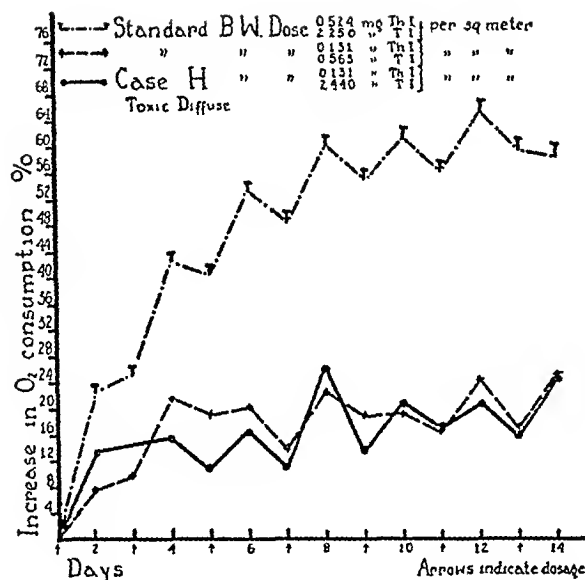


FIG. 7.

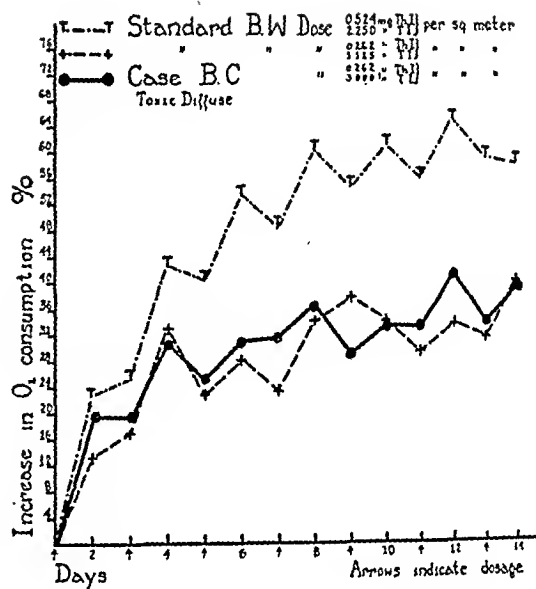


FIG. 8.

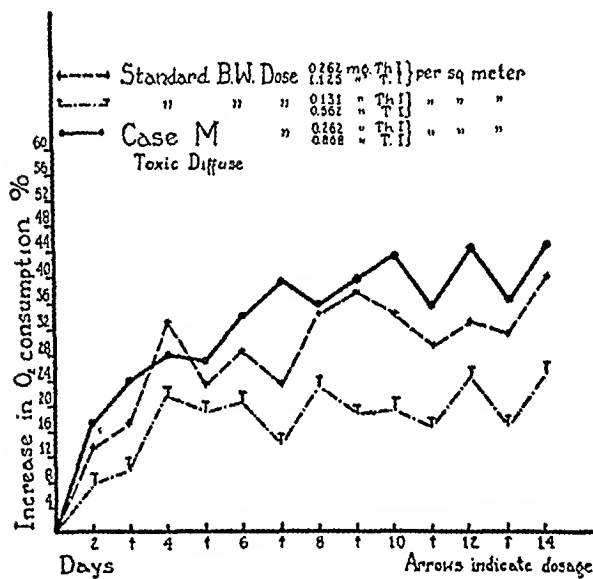


FIG. 9.

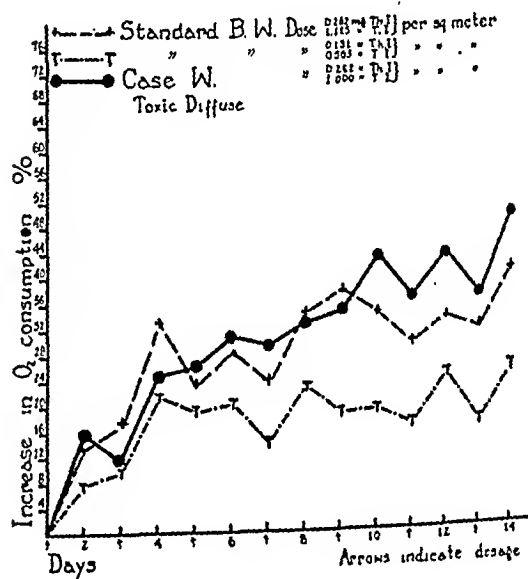


FIG. 10.

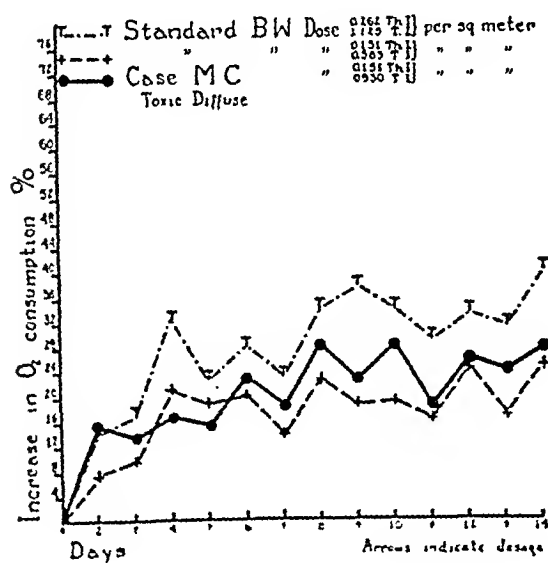


FIG. 11.

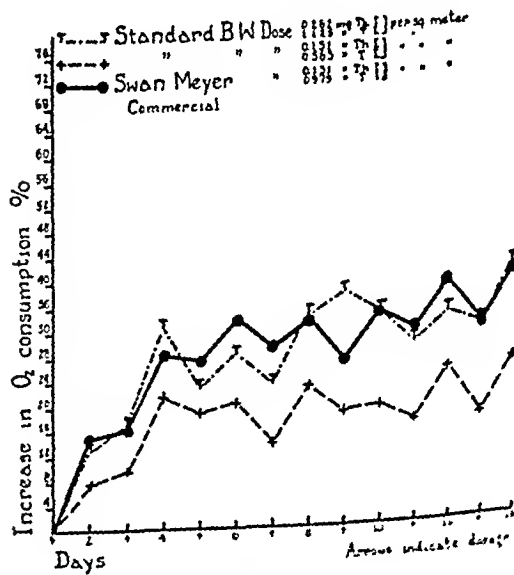


FIG. 12.

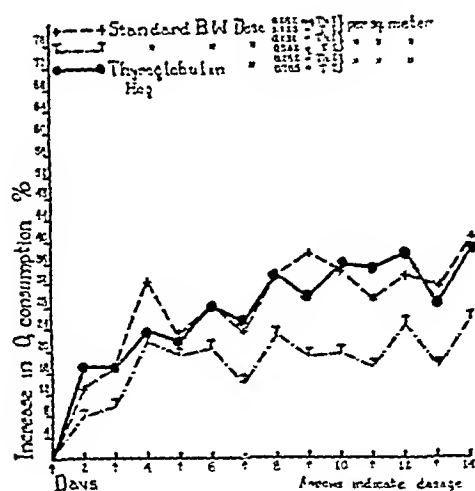


FIG. 13.

FIGS. 3 TO 13, INCLUSIVE. COMPARISONS BETWEEN INCREASES IN OXYGEN CONSUMPTION PRODUCED BY THE STANDARD THYROID PREPARATION, BURROUGHS WELLCOME COMPANY, AND HUMAN THYROID GLAND PREPARATIONS.

Broken line (---) represents the increase in oxygen consumption observed after administration of an amount of the standard thyroid preparation containing the same dosage of thyroxine iodine as of the unknown preparation (—) given. Broken line (---) represents the increase in oxygen consumption of the standard after administration of a dose containing an amount of total iodine approximately equivalent to that in the unknown preparation. Standard curves are taken from Figure 1. Points are obtained by averaging the increase in oxygen consumption of all of the animals of a group.

TABLE III  
Effects of administration of desiccated thyroid, thyroglobulin and thyroxine to guinea pigs

Preparation	Animal number	Increase in O <sub>2</sub> consumption		Mean increase in O <sub>2</sub> consumption resulting from administration of standard, Burroughs Wellcome		Average weight loss on 14th day with unknown preparations
		Mean of last 3 days of individual animals	Mean of group	In equithyroxine I dosage	In equitotal iodine dosage*	
Case 9. Normal.....	70	per cent	per cent	per cent	per cent	per cent of average initial body weight
	73	30.7				
	74	48.3				
	75	34.9				
	76	34.6	31.5	35.3	64.0	
	77	27.9				
	78	21.2				
Case 11. Normal.....	63	23.0				8.0
	65	39.7				
	66	43.7				
	67	36.6	36.3	35.3	29.0	
	68	31.8				
	69	34.2				
Swan-Meyer.† Commercial.....	112	31.8				12.7
	113	31.9				
	114	33.6				
	115	38.3	36.6	22.2	33.0	
	116	40.7				
	117	40.6				

\* Each figure represents the increase in O<sub>2</sub> consumption which would be expected to result from a dose of Burroughs Wellcome, standard, containing the same amount of total iodine as the dose of the unknown preparation given. Figures are interpolated from the standard curve (Figure 2).

† Experiment was repeated with a second group of animals—similar results were obtained.



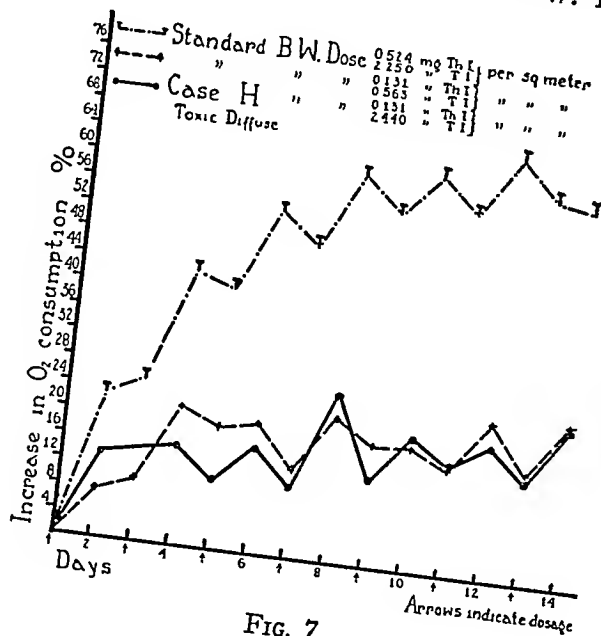


FIG. 7.

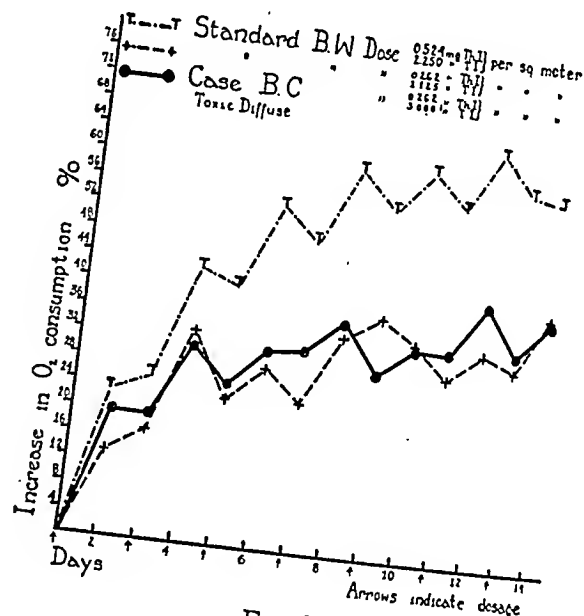


FIG. 8.

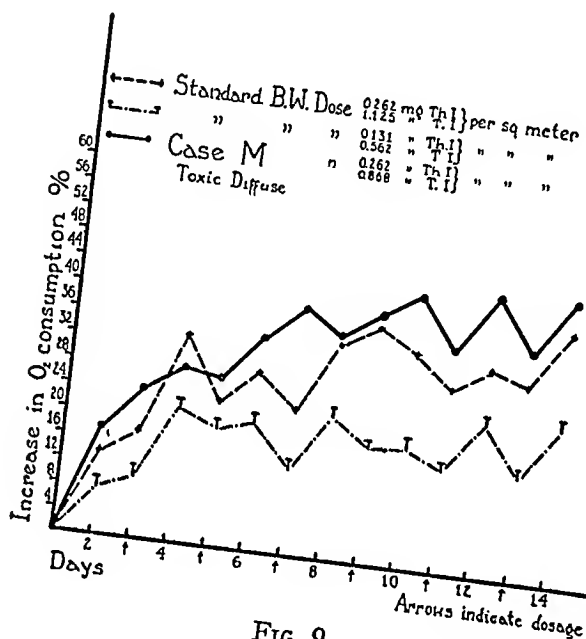


FIG. 9.

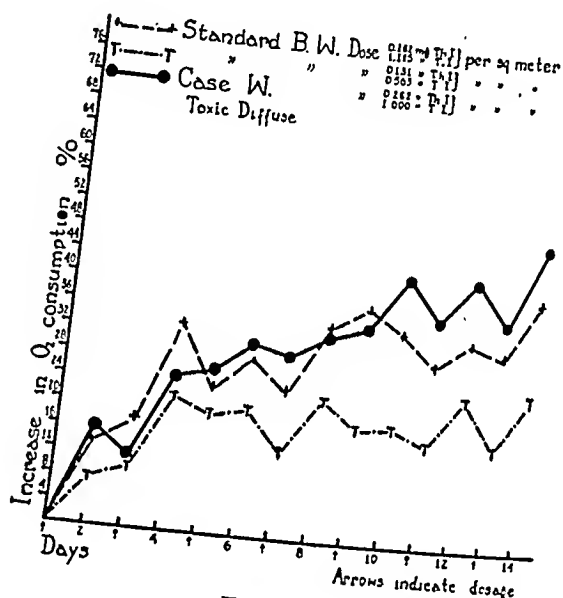


FIG. 10.

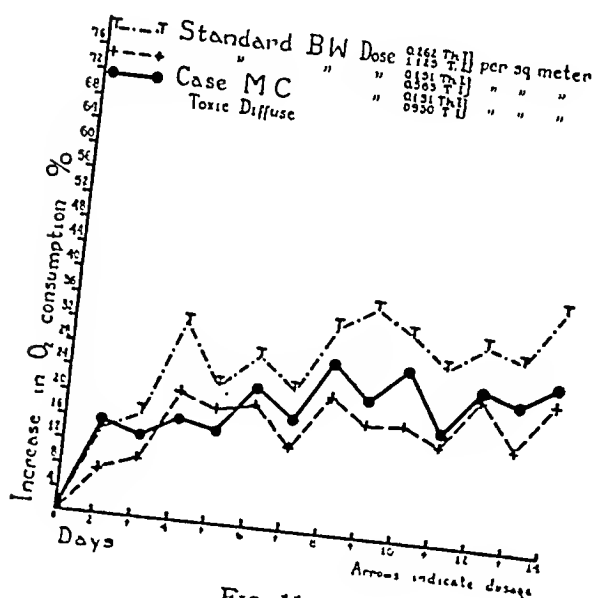


FIG. 11.

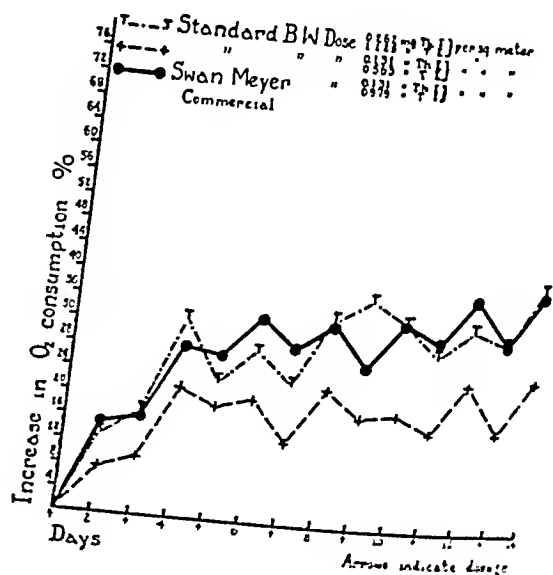


FIG. 12.

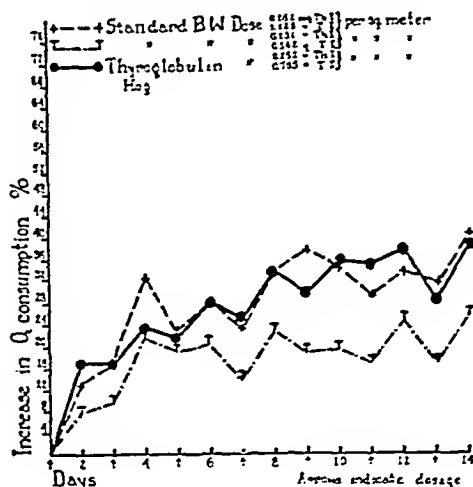


FIG. 13.

FIGS. 3 TO 13, INCLUSIVE. COMPARISONS BETWEEN INCREASES IN OXYGEN CONSUMPTION PRODUCED BY THE STANDARD THYROID PREPARATION, BURROUGHS WELLCOME COMPANY, AND HUMAN THYROID GLAND PREPARATIONS.

Broken line (---) represents the increase in oxygen consumption observed after administration of an amount of the standard thyroid preparation containing the same dosage of thyroxine iodine as of the unknown preparation (—) given. Broken line (— · — ·) represents the increase in oxygen consumption of the standard after administration of a dose containing an amount of total iodine approximately equivalent to that in the unknown preparation. Standard curves are taken from Figure 1. Points are obtained by averaging the increase in oxygen consumption of all of the animals of a group.

TABLE III  
*Effects of administration of desiccated thyroid, thyroglobulin and thyroxine to guinea pigs*

Preparation	Animal number	Increase in O <sub>2</sub> consumption		Mean increase in O <sub>2</sub> consumption resulting from administration of standard, Burroughs Wellcome		Average weight loss on 14th day with unknown preparations
		Mean of last 3 days of individual animals	Mean of group	In equithyroxine I dosage	In equitotal iodine dosage*	
		per cent	per cent	per cent	per cent	per cent of average initial body weight
Case 9. Normal.....	70	30.7	31.5	35.3	64.0	8.0
	73	48.3				
	74	34.9				
	75	34.6				
	76	27.9				
	77	21.2				
	78	23.0				
Case 11. Normal.....	63	39.7	36.3	35.3	29.0	9.5
	65	43.7				
	66	36.6				
	67	31.8				
	68	34.2				
	69	31.8				
Swan-Meyer.† Commercial.....	112	31.9	36.6	22.2	33.0	12.7
	113	33.6				
	114	38.3				
	115	40.7				
	116	40.6				
	117	34.3				

\* Each figure represents the increase in O<sub>2</sub> consumption which would be expected to result from a dose of Burroughs Wellcome, standard, containing the same amount of total iodine as the dose of the unknown preparation given. Figures are interpolated from the standard curve (Figure 2).

† Experiment was repeated with a second group of animals—similar results were obtained.

TABLE III—*Continued*

Preparation	Animal number	Increase in O <sub>2</sub> consumption		Mean increase in O <sub>2</sub> consumption resulting from administration of standard, Burroughs Wellcome		Average weight loss on 14th day with unknown preparations
		Mean of last 3 days of individual animals	Mean of group	In equithyroxine I dosage	In equitotal iodine dosage*	
Case P. Non-toxic nodular goiter.....	29	<i>per cent</i> 22.1	22.0	<i>per cent</i> 22.2	<i>per cent</i> 47.0	<i>per cent of average initial body weight</i> 7.2
	30	20.0				
	32	23.6				
	33	22.3				
	34	25.9				
	35	17.9				
Case R. Non-toxic nodular.....	38	23.1	26.4	22.2	53.0	4.4
	44	38.4				
	46	18.3				
	47	24.6				
	48	24.2				
	49	30.0				
Case H. Toxic diffuse goiter.....	72	22.7	20.2	22.2	65.0	2.4
	80	17.4				
	81	21.4				
	82	20.8				
	83	22.5				
	84	16.1				
Case B.C. Toxic diffuse goiter.....	85	34.7	38.3	35.3	65.0+	5.9
	86	39.8				
	87	41.7				
	88	36.5				
	89	40.7				
	90	36.2				
Case W. Toxic diffuse goiter.....	91	27.9	43.3	35.3	33.0	8.7
	94	44.1				
	95	42.7				
	96	62.4				
	97	36.7				
	98	46.1				
Case M.† Toxic diffuse goiter.....	100	37.9	42.1	35.3	30.0	12.5
	101	57.4				
	103	42.0				
	104	45.2				
	105	28.2				
Case M. C.† Toxic diffuse goiter.....	106	29.9	26.1	22.2	31.0	8.2
	107	28.1				
	108	25.0				
	109	30.4				
	110	30.4				
	111	12.9				
Pure thyroxine as di-sodium salt by mouth	16	23.2	After 14 days 17.9	35.3		0.0
	18	8.6				
	19	21.3				
	20	21.1				
	21	14.8				
	22	18.4				
Thyroglobulin. Hog.....	118	28.8	34.8	35.3	27.0	11.8
	119	49.3				
	120	36.7				
	122	25.0				
	123	34.0				

thyroxine iodine. If, however, the calorigenic effect were proportional to the total iodine, the increase in oxygen consumption would approximate an equi-total iodine dosage of the standard. As will be seen from a study of these curves, with the exception of one case, a satisfactory correlation was observed between the calorigenic effect and the thyroxine iodine content of the series of preparations.

The glands from Cases 9 and 11 (Figures 3 and 4) are human thyroids removed from apparently normal individuals who had met with sudden traumatic death. Histological examination showed the tissue to be normal. The curve of Case 9 follows closely the thyroxine iodine equivalent curve of the standard, and the results of this experiment, therefore, are strikingly in favor of the thyroxine iodine as a measure of the calorigenic activity of the preparation. On the basis of the total iodine content of the dose administered (2.3 mgm.) an increase in the oxygen consumption of more than 61 per cent (average of the last 3 days) would have been expected. An average increase of 31.5 per cent was obtained, which agrees with the thyroxine iodine equivalent of the standard 35.3 per cent sufficiently well for this type of work. A better agreement, 36.3 per cent, was obtained with Case 11 (Figure 4), although the results as a whole are not as striking since the total iodine content did not differ as widely from the standard. If the activity were proportional to the total iodine the curve should fall in this case between the two standard curves shown.

Figures 5 and 6 (Cases P and R) show results obtained with two non-toxic nodular glands. There can be no doubt in either of these cases that the activity parallels the thyroxine fraction of the total iodine.

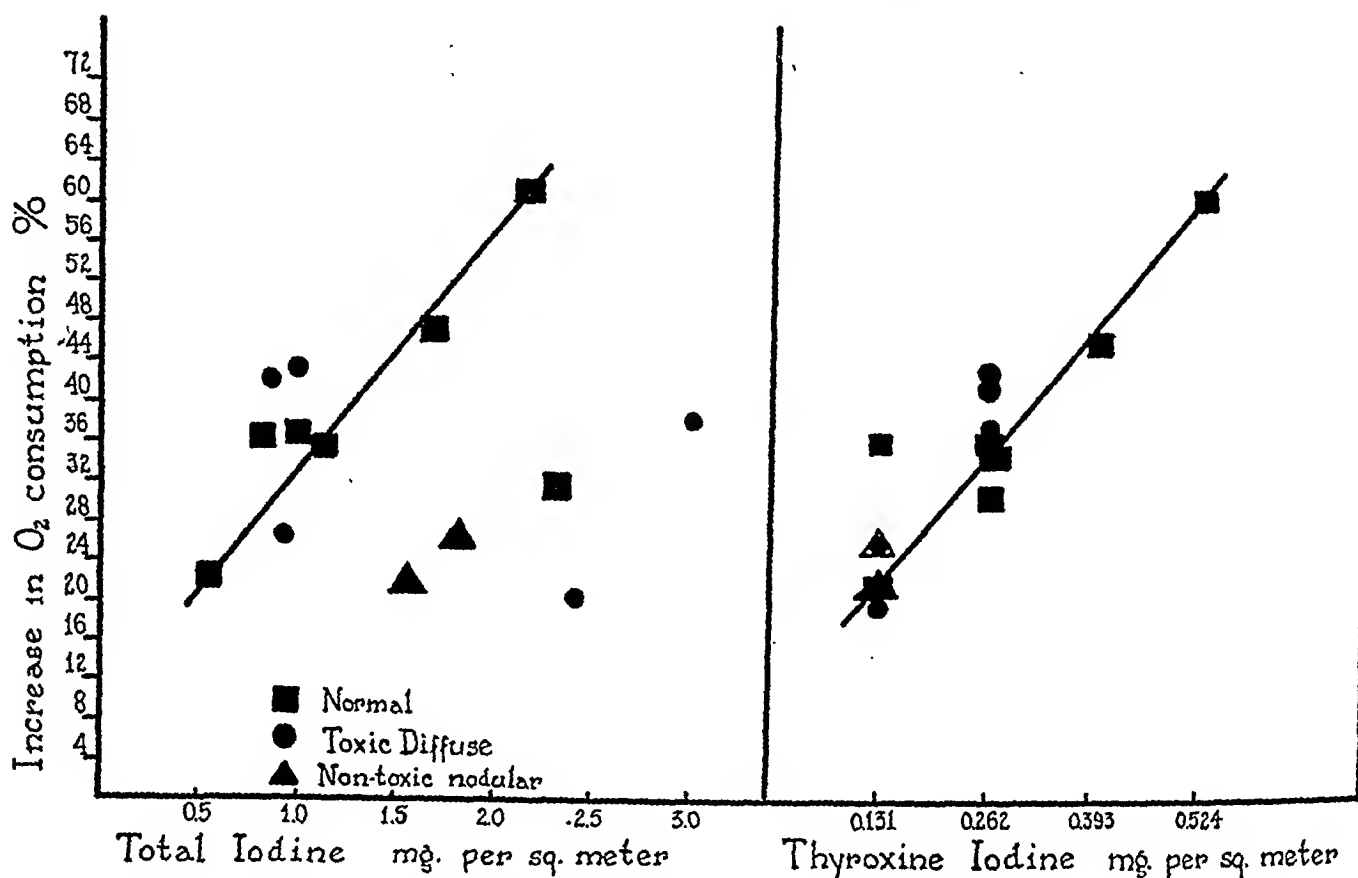
The calorigenic effect of the preparations from cases of toxic diffuse goiter, H, B.C., M, W and M.C., are shown in Figures 7, 8, 9, 10 and 11 respectively. In the two preparations from toxic diffuse glands, Cases H and B.C., plotted in Figures 7 and 8, the increase in oxygen consumption is in striking agreement with the standard based on equi-thyroxine dosage, notwithstanding the large non-thyroxine iodine moiety in these two glands. The preparations from Cases M, W, and M.C.,

also toxic glands, recorded in Figures 9, 10 and 11, caused a slightly greater increase in oxygen consumption than might be expected when compared with the standard. In each of the two animal groups to which preparations from Cases M and W were administered there was a hypersensitive animal which raised the average (see Table III). Discrepancies of this type are to be expected with the method employed.

The commercial thyroid preparation of Swan-Meyer is included in the series (Figure 12). The increase in oxygen consumption observed when this preparation was given to six guinea pigs is higher than would be expected on the basis of its thyroxine content as determined by chemical analysis. Since this was the only preparation which proved to be inconsistent with the thyroxine-oxygen consumption relationship the experiment was repeated and the same result was obtained. While the unbroken line of Figure 12 should fall on the lowest standard curve in order to regard the activity as parallel to the thyroxine content, it actually falls above the theoretical total iodine curve. It is possible that some "filler" was present in this commercial preparation which interfered with the correct determination of thyroxine. An effort was made to obtain more material for further chemical investigation but unfortunately the company had ceased to make the product.

A comparison was made between the standard and a thyroglobulin preparation kindly supplied by Dr. M. Heidelberger. This material had not been submitted to any process of drying. The increase in oxygen consumption observed (Figure 13) was practically identical with the equi-thyroxine dose of the standard. From this it was concluded that the process of drying employed for our desiccated thyroid material had not decreased the biological activity.

A summary of the results of this investigation is shown in Figure 14. When the increase in oxygen consumption is plotted against the total iodine content of the dose of thyroid administered, the results show a marked scatter. However, when plotted against thyroxine content, the results (with the exception of one mentioned above) are found to group themselves closely about the points representing equivalent amounts of thyroxine iodine in the standard. From these



CALORIGENIC EFFECT OF NORMAL AND PATHOLOGICAL THYROID GLAND PREPARATIONS WHEN ADMINISTERED IN EQUI-IODINE DOSES.

CALORIGENIC EFFECT OF NORMAL AND PATHOLOGICAL THYROID GLAND PREPARATIONS WHEN ADMINISTERED IN EQUI-THYROXINE IODINE DOSES.

FIG. 14.

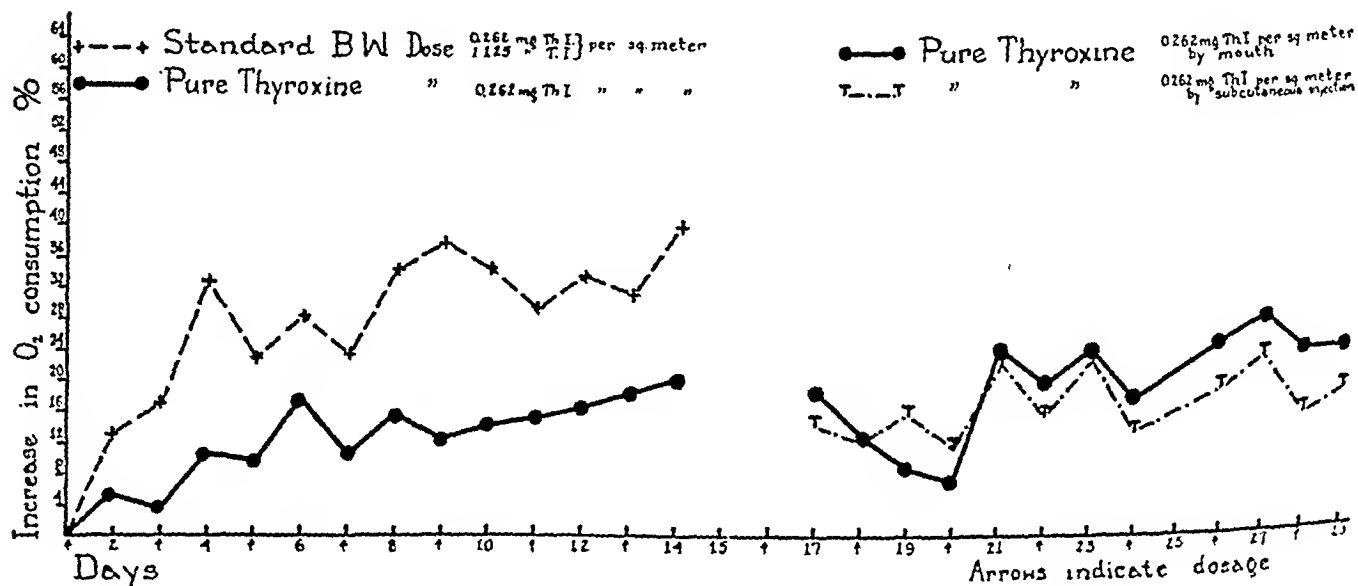


FIG. 15. COMPARISON BETWEEN INCREASES IN OXYGEN CONSUMPTION PRODUCED BY STANDARD, BURROUGHS WELLCOME COMPANY THYROID, AND THE DI-SODIUM SALT OF RACEMIC THYROXINE WHEN ADMINISTERED IN EQUI-THYROXINE IODINE DOSES.

results it seems reasonable to conclude that the calorogenic activity of desiccated thyroid preparations is proportional to the thyroxine iodine content as determined chemically rather than to the total iodine content of the gland.

A comparison (Figure 15) was made between the standard and pure crystalline thyroxine<sup>1</sup> in alkaline solution. After 14 days of dosage a mean increase of only 17.5 per cent in the oxygen consumption was observed as compared with 35.3 per cent obtained with an equi-thyroxine dose of the standard. To eliminate the possibility of failure of absorption, two of the six animals of the group were chosen as controls and were given the same dose by mouth over a second 14 day period while the remaining four were injected subcutaneously. At the end of the second 14 day period (total of 28 days) the two animals receiving the dose by mouth showed an increase in oxygen consumption of from 17.5 to 25 per cent, while those who had been injected had only increased from 17.5 to 18.5 per cent. In other words, the dose when given by mouth had been as effective as when administered parenterally, therefore the fact that only 50 per cent as much effect was obtained as in the case of the standard could not be attributed to lack of absorption.

#### DISCUSSION

An analysis of the data presented above permits of a reasonably definite answer to the first question proposed. With one exception, normal and pathological thyroid preparations when administered to normal guinea pigs increased the oxygen consumption in proportion to the amount of thyroxine contained therein. No correlation between the total iodine of the glands and increase in metabolic rate was observed. The one preparation not consistent with the other eleven does not, it is believed, invalidate the conclusion reached. As mentioned above the preparation was a commercial product, and the possibility of error in the chemical determination of thyroxine could not be ruled out on account of lack of material.

<sup>1</sup> Thyroxine was isolated from Burroughs Wellcome Company thyroid by extraction of alkaline hydrolysate with butyl alcohol. The iodine content of this preparation was 63.1 per cent as determined by Pregl's method. Calculated 65.4 per cent.

The experiments bring forward no evidence for or against the postulate of "active iodine" not in the form of thyroxine. It is possible that there may exist within thyroid glands, both normal and pathological, so called "active iodine" not in the form of thyroxine but, if this be so, such iodine would appear, from the results obtained, to exist in amounts proportional to the amount of thyroxine present.

Because of the length of time required for a single experiment, the preparations selected for study are not large in number. They include, however, a sufficiently wide range of the normal and pathological human glands to bring out differences should such exist. The non-iodized toxic diffuse gland is, unfortunately, not included in the series. As much as this type of gland is desired, it was not considered right or proper to request a surgeon to operate on a toxic patient without previous administration of iodine. Whether the same relationship between the thyroxine content of the non-iodized toxic glands and oxygen consumption pertains as in iodized glands is not known.

Is there an abnormal (17) substance more toxic than the normal hormone present in the toxic goiter, particularly the toxic diffuse gland? From the evidence available the question may be answered in the negative. Certainly, so far as the calorogenic activity of the glands is concerned, there is no difference to be observed between normal, non-toxic and toxic glands. Particular attention to toxic symptoms during the administration of preparations from toxic glands revealed no differences in toxic and non-toxic glands. Evidence against the presence of an abnormal toxic substance in the toxic diffuse goiters would be more conclusive were it possible to report observations on a non-iodized toxic gland.

The method selected for the biological assay of the thyroid gland preparations appeared to be the most suitable in the circumstances. On account of the well known variability among clinical conditions it was considered highly desirable to use separate individual glands and not to employ combinations. Small animals were necessary for this reason. Normal instead of thyroidectomized animals were chosen. On theoretical grounds, perhaps, thyroidectomized animals might be preferred. The experience of Gaddum (18), to-

gether with a preliminary observation in our laboratory, indicates that this is not the case. It is true that human subjects with spontaneous myxedema are more sensitive to the administration of thyroid gland than are normal individuals. Aside from the increased sensitivity of myxedematous patients there is no evidence that the effect of the thyroid hormone is in any way different from normal. In both animals and human subjects, either with hypothyroidism or without, there is considerable variability in response to the administration of thyroid gland. In the experimental work, the attempt has been made to compensate for this variation by including at least six animals for a given preparation, and calculating the increase in oxygen consumption from the normal of each animal rather than the group normal average.

The discrepancy between the calorogenic activity observed with crystalline racemic thyroxine administered as the di-sodium salt may be accounted for in large part by the relatively low activity of the racemic compound as compared with l-thyroxine, the form in which the hormone probably exists in the gland (19). Gaddum (18) has found the l-form to be three times as active as the d-form, which indicates that an equi-thyroxine iodine dose of the dried thyroid standard should show 1.5 times as much activity as racemic thyroxine. Activity two times as great was actually found, a result that only approximates the expected value. In this connection, it should be mentioned that Salter, Lerman and Means (20) found no difference in the calorogenic activity of the d- and l-forms of thyroxine when administered to patients with spontaneous myxedema.

The results tabulated in Table III are in general agreement with those found by Gaddum and Hetherington (9), Rotter and Mecz (10), and Sjögren and Lundgren (11) when different biological methods were used. The discrepancy between the conclusions drawn from this investigation and those of Hunt and Seidell (1) and of Krogh and Lindberg (2) may be apparent rather than real. As the preparations used by these workers were not analyzed for their thyroxine iodine content the possibility exists that the thyroxine per cents of total iodine may have been so nearly alike that activity proportional to total iodine might also be proportional to thyroxine

iodine. The results of this study are at variance with the work of Means, Lerman and Salter (3) (21). The method of biological assay used in our studies is different from that employed by these investigators and may account for the difference in results obtained.

#### SUMMARY

1. The calorogenic effects on normal guinea pigs of twelve preparations of thyroid glands in equi-thyroxine doses have been studied. These preparations consisted of two commercial thyroid gland products, one preparation of hog thyroglobulin, and nine human thyroid glands two of which were normal, two non-toxic and five toxic diffuse.

In addition, the calorogenic effect of racemic thyroxine administered as the di-sodium salt was studied.

2. The results obtained indicate that the calorogenic activity of desiccated thyroid preparations is proportional to the thyroxine content of the gland as determined by the method of Leland and Foster.

3. The calorogenic activity of crystalline racemic thyroxine administered as the di-sodium salt was only 50 per cent as great as an equi-thyroxine dose of thyroid gland. It is suggested that this reduced effect may be explained by the greater activity of l-thyroxine, the form in which the hormone exists in whole gland.

4. No evidence was obtained in favor of the existence of an abnormal substance contributing to the toxicity of pathological glands.

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# CLINICAL OBSERVATIONS ON THE EVENTS PRECEDING THE APPEARANCE OF RHEUMATIC FEVER<sup>1, 2</sup>

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The frequent association between tonsillitis and upper respiratory tract infection and subsequent rheumatic fever has been observed and commented upon by many students of the disease. It is generally agreed that this relationship between the preceding infection and subsequent rheumatic fever is too consistent to be merely a chance occurrence. This important phase of the natural course of rheumatic fever has been commented upon by Coburn (1, 2), Schlesinger (3), Collis (4), Sheldon (5), Gibson, Thomson, and Stewart (6), Boas and Schwartz (7), Hiller and Graef (8), and others. In general, we agree with these investigators both as regards the association between infection of the upper respiratory tract and rheumatic fever, as well as to apparent epidemics of rheumatic fever, following prevalent respiratory infection in groups of closely associated individuals. The percentage of instances of rheumatic fever preceded by recent upper respiratory infection may be as high as 75 per cent. Not only does this relationship hold for the initial appearance of the disease, but also for subsequent recurrences or recrudescences. It is of further interest that in most instances there exists, as pointed out by Schlesinger (3), a well defined latent period varying from a few days up to four weeks between the febrile respiratory tract infection and the occurrence of the clinical signs and symptoms of rheumatic fever. Less frequently the disease appears to be ushered in or occurs concurrently with the respiratory infection.

Because of the obscure nature of the etiological factors involved, attention has been directed, and we believe rightly, to this so-called respiratory phase of the disease and the subsequent latent period. Its analogy to the sequence of events occurring in serum sickness and other frankly al-

lergic conditions has been commented upon by Zinsser (9), Swift (10), and others. Exhaustive study has been centered upon the cultural characteristics of the respiratory flora during these preceding infections in an attempt to establish a specific causative agent. In so far as we are aware no proof has yet been presented that any organism found in the upper respiratory tract is the cause of rheumatic fever.

It is the purpose of this paper to present certain clinical observations on rheumatic fever which are of interest and may be of importance in future considerations of possible factors in the causation of the disease. During the past thirteen years at the House of the Good Samaritan there has been an opportunity to observe the clinical course of a large number of children and adolescents (over 1,200) during various phases of rheumatic fever in a hospital where they receive bed care until all clinical and laboratory evidence of infection has subsided. The average period of observation in the hospital is four months, but not infrequently is as long as several years. During this period of prolonged observation, the evidence of persistent low-grade and sub-clinical infection has been largely that of a slight fever (rectal), subcutaneous nodules, erythema multiforme, a moderate leukocytosis, increased sedimentation rate of the red blood cells, or prolongation of the auriculoventricular conduction time. It is in this mildly active group that we have observed repeatedly the importance of respiratory infection in precipitating relapses or recrudescences of the clinical manifestations of the acute disease. Figure 1 shows the bedside chart of a boy, aged thirteen, convalescing from recent rheumatic fever in whom the clinical and laboratory evidence of active disease had subsided. Following the febrile respiratory infection shown on the chart and after a latent period of two weeks, the boy developed fulminant rheumatic fever with pericarditis and congestive failure. He died at the end of one

<sup>1</sup> Presented at the meeting of the American Society for Clinical Investigation, Atlantic City, April 30, 1934.

<sup>2</sup> The expenses of this study have been defrayed by a grant from The Commonwealth Fund.

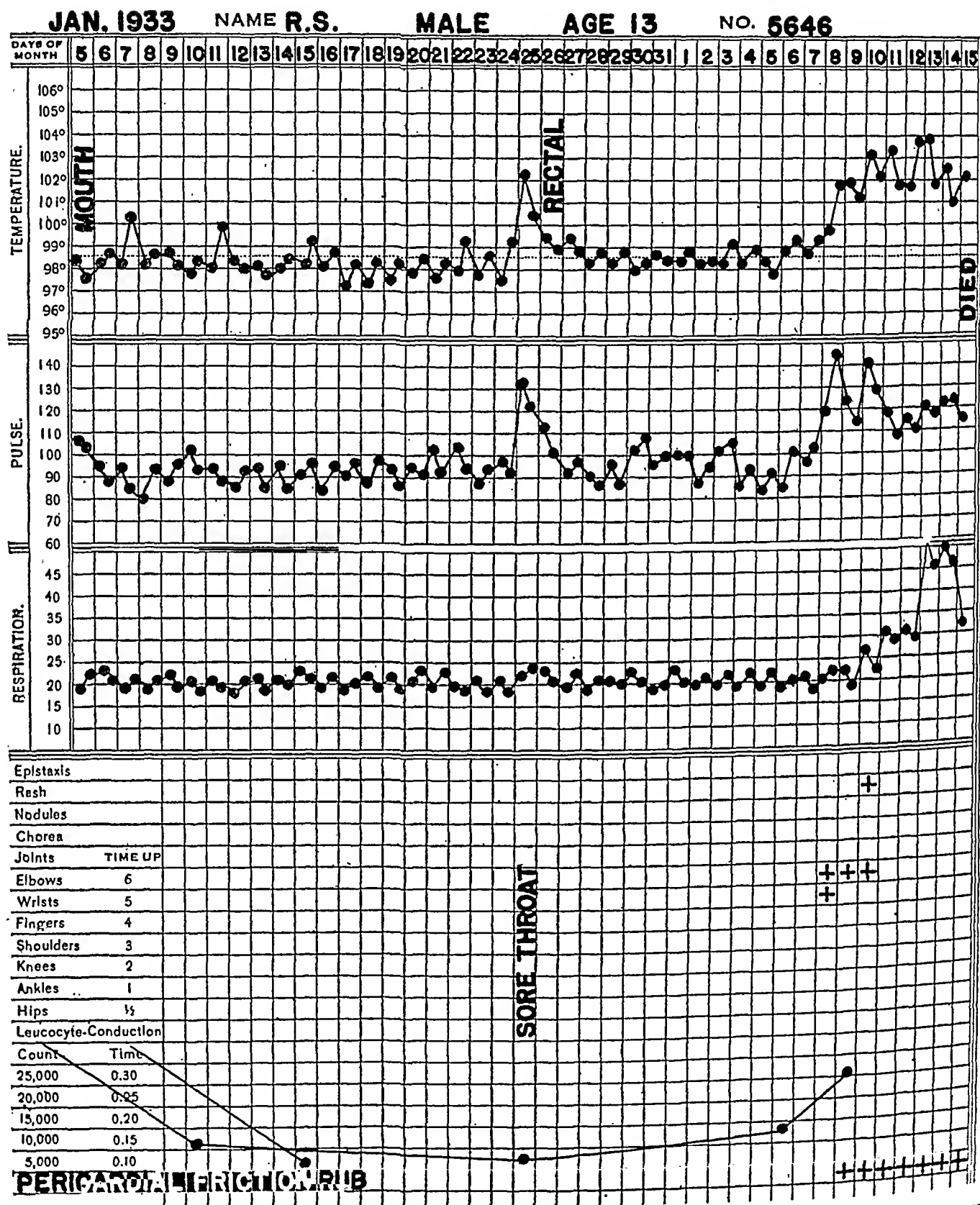


FIG. 1. BEDSIDE CHART SHOWING THE RELATIONSHIP BETWEEN UPPER RESPIRATORY INFECTION AND A SUBSEQUENT RECRUDESCENCE OF FULMINANT RHEUMATIC FEVER.

week, and rheumatic fever was confirmed by autopsy.

Recurrent rheumatic fever has been observed at times following infections other than tonsillitis or pharyngitis. Scarlet fever apparently is frequent as a precipitating factor and further data on this interesting relationship will be published subsequently. These episodes are closely related to streptococcus infection. Instances of other precipitating infections, frequently associated with or subsequent to ordinary respiratory infection noted in our series are: "pneumonia" 4, otitis media 5, measles 3, and erysipelas 1. One might add here single episodes, seemingly independent of respiratory infection, which we have observed to precede recurrent rheumatic fever; namely, pyelitis, herpes zoster, and a febrile reaction to the Schick test.

We have been impressed by the occurrence, now and then, of an equally striking though less frequent association between other (non-infectious) and apparently non-specific episodes and subsequent recurrent rheumatic fever. These episodes exhibit a suggestively similar relationship to the occurrence of subsequent rheumatic fever as has previously been noted between respiratory infection and the acute phase of the disease. Subsequent observations herewith recorded appear to throw additional light upon this interesting but imperfectly understood phenomenon.

#### *Transient and unexplained temperature*

When recrudescences followed respiratory infection, a mild fever of two or three degrees at the time of the infection has been the rule. In contrast, however, we have seen on several occasions a transient fever of two or three degrees for a few hours, unassociated with evident infection or other manifest cause and apparently precipitating after the usual "latent period" a recrudescence of typical rheumatic fever. Figure 2 shows the course of the disease in a child, four years of age, who appeared to be convalescing satisfactorily from rheumatic fever which began twelve months previously. On February 26, as indicated in the chart, there occurred a transient and entirely unexplained elevation of the temperature and pulse for a few hours, followed in two weeks by fulminant rheumatic fever associated with pericar-

ditis, congestive failure and death eleven days after the onset. The response of the temperature to salicylates without clinical improvement in the patient is of interest. Postmortem examination confirmed the clinical impression of severe and fulminant rheumatic fever. In the light of previous observations on the apparent relationship of respiratory infection to acute rheumatic fever the association here seems equally striking, but in this instance recrudescence followed a temperature reaction alone. The relationship appears even more convincing when we consider that the bedside chart for the preceding six months had shown a consistently normal temperature and steady pulse rate.

#### *Accidents and operations*

In our experience this same sequence of events has been precipitated apparently by accidents involving fractured bones or sprained joints. Surgical operations have also appeared to reactivate, occasionally, a latent rheumatic infection. It is not an infrequent experience in general hospitals to observe the recurrence of the signs and symptoms of rheumatic fever following tonsillectomy. Indeed, on rare occasions, the initial attack of rheumatic fever is observed following this procedure. We have rarely encountered such a series of events (in 7 instances), since it has been our policy to delay tonsillectomy until all clinical and laboratory evidence of active rheumatic fever has disappeared. This precaution is probably responsible for the small number of recurrences that we have observed after tonsillectomy. The explanations for the reappearance of rheumatic fever following tonsillectomy have been many and inconclusive. Appendectomy has seemingly been related in 10 instances to a subsequent attack of rheumatic fever. Frequently the appendix, at the time of removal, has appeared to be normal. It may be that some of these cases, at least, exemplify an onset of rheumatic fever with abdominal symptoms rather than a true initiation or reactivation of the rheumatic process by the surgical procedure. Of this we have no proof.

The clinical course of the disease as illustrated in Figure 3 is of unusual interest in this connection. A girl of thirteen years had had four pre-





view of the entire absence of infection either in the respiratory tract or elsewhere.

There remains to be recorded a crucial observation on a girl aged twenty years who entered the medical wards (E. M. 319,881) of the Massachusetts General Hospital with severe chorea and minimal rheumatic heart disease. For the preceding six months she had been in relatively poor health, with vague and repeated joint pains and occasional nosebleeds, prior to the onset of chorea two months before entry. Because of the increasing severity of the chorea, in spite of rest in bed and sedatives, she was given protein shock therapy. After the third daily injection of 0.1 cc. of stock typhoid-paratyphoid vaccine intravenously, she exhibited an unusual and alarming rise in temperature to 108.9° F. (rectal), which subsided promptly after ice packs had been applied. No further immediate complications appeared, and the severity of the chorea was considerably reduced. Two weeks after the last protein shock the patient developed a low-grade fever of from two to three degrees, complained of multiple joint pains, and an aortic diastolic murmur, not previously noted, was heard. The electrocardiogram, taken for the first time, showed a delay in auriculoventricular conduction with a P-R interval of 0.30 second. The joint pains and fever responded promptly to salicylates, and the patient left the hospital against advice. Twelve months later she reported at our request for a follow-up examination. She was in good health and had had no further recurrences of rheumatic fever. There was evidence on physical examination of slight cardiac enlargement with minimal disease of mitral and aortic valves. It occurred to us, in retrospect, that perhaps in this instance by reactivation a low-grade rheumatic fever had been brought to a clinical level as a result of the therapeutic injections of typhoid-paratyphoid vaccine.

#### *Reactions after typhoid-paratyphoid vaccine*

Numerous observers have reported favorably upon the use of intravenous typhoid-paratyphoid vaccine (as an agent for non-specific protein shock) in the treatment of a large number and variety of diseases. This procedure is widely used, especially in treating diseases of unknown

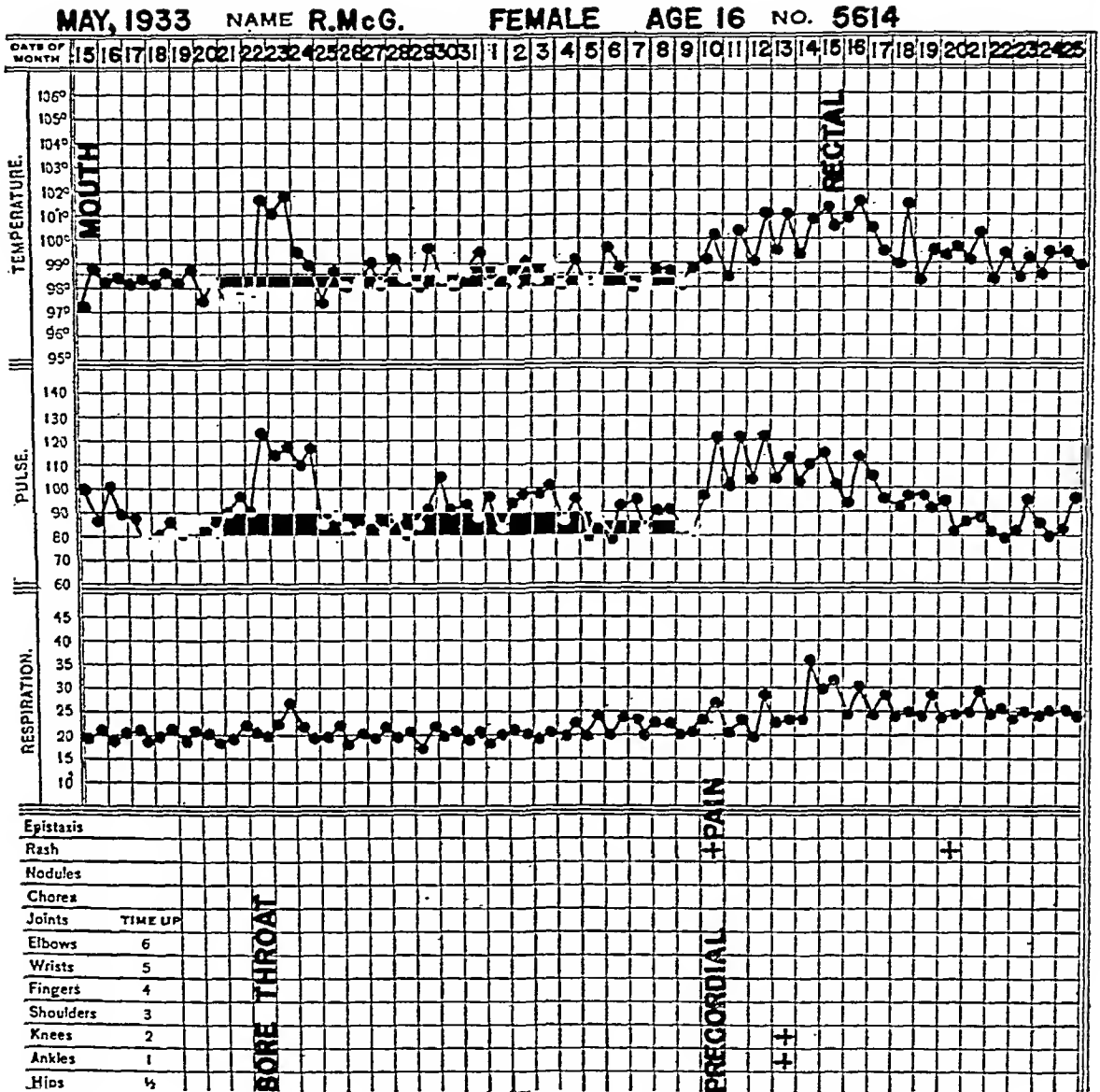
etiology. It seemed logical and justifiable to use this (typhoid-paratyphoid) vaccine intravenously in patients with rheumatic fever in a dosage comparable to that of usual therapeutic procedures. The value of this therapeutic measure has not been completely studied in rheumatic fever. In addition, it was thought possible that one might secure information concerning the rôle of non-specific fever in patients with rheumatic fever, as well as the significance of the febrile episodes often observed before recurrences. The procedure determined upon was to give a single intravenous dose of stock typhoid-paratyphoid vaccine (0.1 cc. of vaccine containing 250,000,000 organisms). This quantity usually results in a moderate temperature reaction and a chill. The method of repeated doses of typhoid-paratyphoid vaccine, as used by Sutton and her co-workers in the treatment of chorea (11) and as has been employed in rheumatic fever (12), has not been followed.

The patients chosen for this procedure were carefully selected. They were chiefly adolescent individuals who had shown no tendency to progressive cardiac disease in spite of frequent attacks of rheumatic fever. Most of the cases had had a number of attacks during a long period of observation. In addition, all were in excellent general physical condition but exhibited evidence, chiefly from laboratory examination, of low-grade chronic rheumatic fever. Save for recent joint pain, occasional epistaxis and an occasional transient erythema marginata in three cases, the evidence of continued rheumatic fever was based entirely on abnormal laboratory findings. A few, as will be pointed out later, had shown in the past a clear relationship between respiratory infection and a subsequent attack of rheumatic fever. We desire to record in some detail the results of these few observations.

#### *Case I*

R. M., H.G.S. 5614, female, age 16, gave a history of frequent attacks of rheumatic fever since the age of six years, usually following a cold but on two occasions after pneumonia. On entrance to the hospital, in November, 1932, she had laboratory evidence of low-grade rheumatic infection with moderate enlargement of the heart and the murmurs of mitral stenosis and regurgitation and slight aortic regurgitation.

*Observation I.* In May, 1933, Figure 4, she developed



a sore throat and fever for two days. After a latent period of three weeks a recrudescence of clinically active rheumatic fever appeared, associated with fever, joint and precordial pain and erythema multiforme. Hemolytic streptococci were present in throat cultures throughout both the respiratory and the rheumatic fever phases of this observation.

*Observation II.* After this episode had subsided she was given, in July, 1933, an intravenous injection of 0.1 cc. of stock typhoid-paratyphoid vaccine, which resulted in a mild and transient temperature reaction as indicated in Figure 5. After a latent period of three weeks a typical recrudescence of clinically active rheumatic fever

appeared with fever, multiple polyarthritides and erythema multiforme. The response to salicylates was striking.

*Observation III.* In January, 1934, a temperature of 103° F. (rectal) was induced artificially for three hours by means of exposure in a hyperthermia box. This treatment was not followed by any evidence, during subsequent observation, of a clinical recurrence of rheumatic fever. Hemolytic streptococci were absent throughout this observation.

**Observation IV.** In February, 1934, and seven weeks after the third observation, the patient received for the second time a single injection of 0.1 cc. of vaccine which precipitated a slight chill and the usual febrile reaction



**JULY, 1933 NAME R.McG. FEMALE AGE 16 NO. 5614**

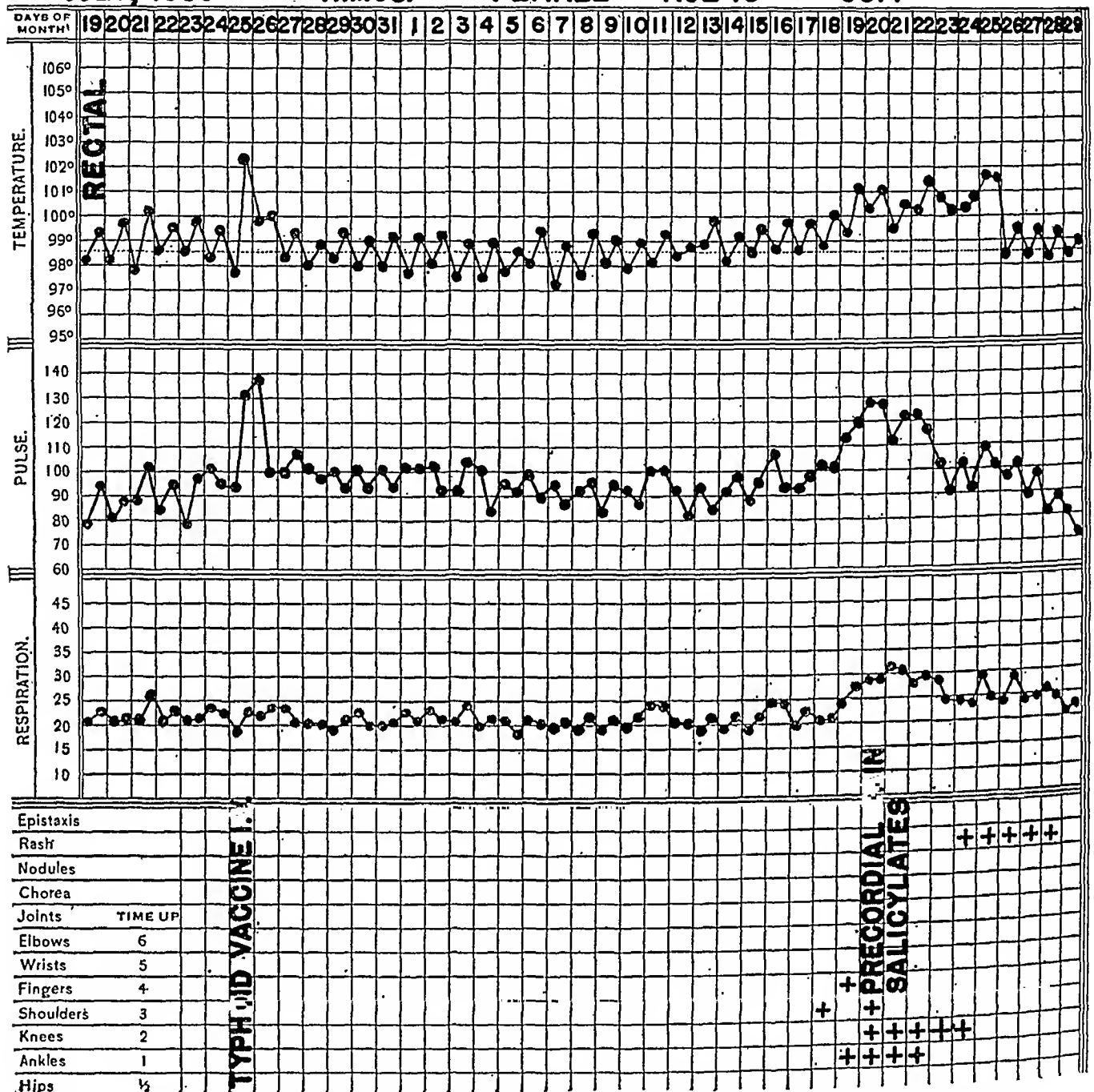


FIG. 5. A RECRUDESCENCE OF RHEUMATIC FEVER FOLLOWING PROTEIN SHOCK IN THE SAME PATIENT AS IN FIGURE 4.

(Figure 6). In contrast to the previous postvaccinal reaction, an immediate reactivation of the latent rheumatic infection became manifest with the appearance of fever, multiple arthritis, erythema multiforme, and an elevation of the corrected sedimentation index (method of Rourke and Ernstene (13)) from a previously normal level of 0.28 mm. per minute to 1.10 mm. per minute, together with an increase in auriculoventricular conduction time from 0.16 second to 0.19 second. The symptoms subsided spontaneously without salicylate therapy. Hemolytic streptococci were absent throughout this observation. Of considerable interest is the fact that our clinical impression suggests that in spite of the apparently in-

duced recrudescence of rheumatic fever this patient's low-grade infection of several years standing was definitely improved following the last episode, so that in May, 1934, all laboratory and clinical evidence of infection had disappeared and she was discharged from the hospital. Her cardiac status remained essentially as at entry.

### Case II

I. P., H.G.S. 5703, female, age 17, gave a history of rheumatic fever at the age of twelve years, with probable low-grade activity during subsequent winters as indicated by frequent joint pains, poor health and rose-

**FEB. 1934**    **NAME R. McG.**    **FEMALE**    **AGE 17**    **NO. 5614**

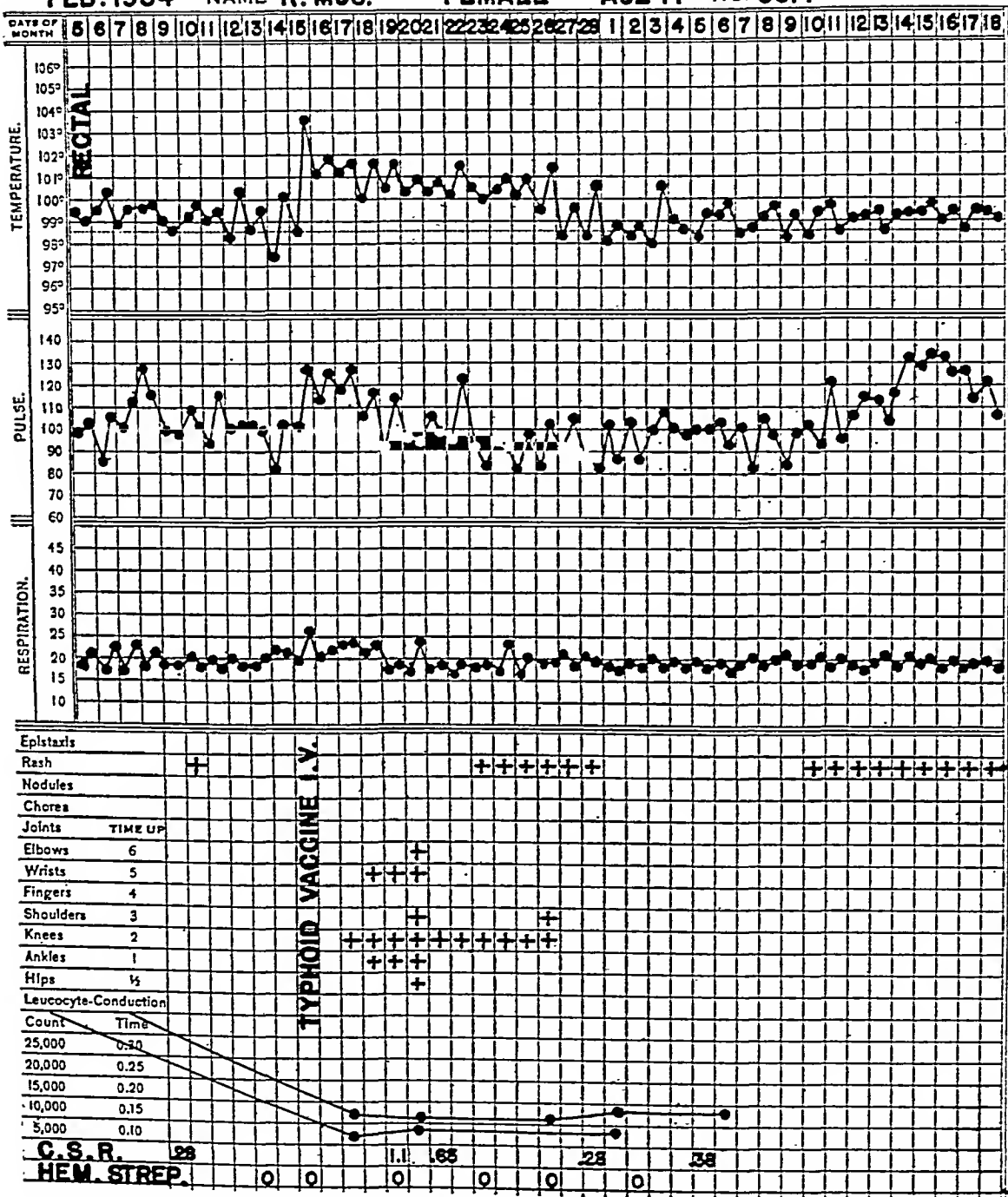


FIG. 6. A SECOND AND IMMEDIATE RECRUDESCENCE OF RHEUMATIC FEVER FOLLOWING PROTEIN SHOCK IN THE SAME PATIENT AS IN FIGURES 4 AND 5.

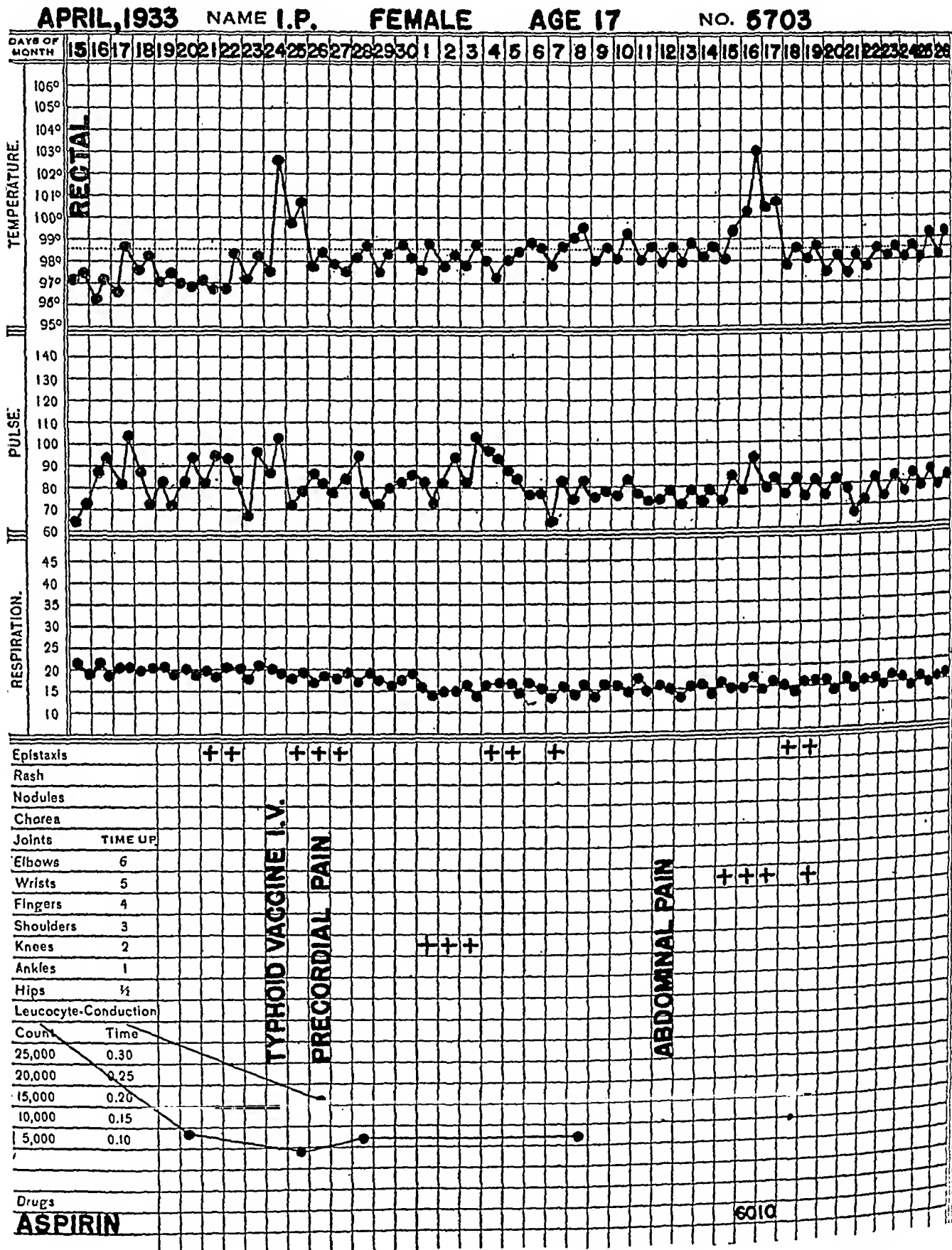


FIG. 7. A RECRUDESCENCE OF RHEUMATIC FEVER THREE WEEKS AFTER A SINGLE PROTEIN SHOCK.

bleeds. In October, 1932, following a tonsillectomy, she had mild rheumatic fever with symptoms for three weeks. In February, 1933, she had a sore throat and the "grippe." Three weeks later she entered the hospital because of general poor condition and was found to have evidence of active rheumatic infection with fever, precordial pain, occasional nosebleeds, and a well marked elevation of the corrected sedimentation index of 1.40 mm. per minute. The heart was moderately enlarged, with the murmurs of mitral regurgitation and stenosis and aortic regurgitation. She improved with rest in bed, and after one week the temperature returned to normal.

*Observation I.* In April, 1933, she was given a single intravenous injection of 0.1 cc. typhoid-paratyphoid vaccine, which was followed by a transient elevation of temperature and a slight chill (Figure 7). During the subsequent three weeks there occurred frequent slight nosebleeds (present before), vague ankle pains, occasional precordial and abdominal pain. At the end of the third week there appeared a frank flare-up with fever and an acutely swollen, red and tender wrist, which became so painful that salicylates were required. Prompt relief resulted, but a vague discomfort in the wrist and fingers persisted for several days. Throat cultures were not done at this time. The patient was discharged from the hospital in November, 1933, without further occurrence of acute rheumatic fever. The heart remained as at entry.

### Case III

E. F., H.G.S. 5876, female, age 16, gave a history of the onset of rheumatic fever and rheumatic heart disease at the age of six years. At the age of thirteen she had a recurrence of rheumatic fever. In January, 1933, at the age of 16, there was a third attack one week after a sore throat, and she entered the hospital for prolonged bed care. She appeared to be in good general condition, and the only evidence of low-grade infection was an occasional nosebleed and almost daily erythema multiforme. The auriculoventricular conduction time by electrocardiogram remained at 0.20-0.21 second.

*Observation I.* In April, 1933, a temperature and associated chill was induced by the intravenous administration of 0.1 cc. of typhoid-paratyphoid vaccine as before. A slight rise in temperature to 100 to 100.5° F. (rectal) persisted for five days, but there was no definite evidence of a subsequent flare-up of the rheumatic process. Hemolytic streptococci were present in throat cultures taken on the day of the protein shock, absent on the fifth day, and again present on the ninth day after the injection. The white blood count and the auriculoventricular conduction time remained unchanged.

*Observation II.* In January, 1934, an artificial fever of 102.5° F. (rectal) induced by means of a hyperthermia box was not followed by any clinical manifestations of rheumatic fever. Throat cultures showed no hemolytic streptococci before the procedure. Hemolytic streptococci were present in the throat on the first day after the treatment, and absent on subsequent days.

*Observation III.* In February, 1934, a single protein shock was induced in the usual manner by the intravenous injection of 0.1 cc. of vaccine (Figure 8). This was followed by a low-grade fever for five days associated with a nosebleed and the reappearance of erythema multiforme on the seventh day, but of most interest, perhaps, was the electrocardiographic evidence of slightly delayed but progressively increasing block in auriculoventricular conduction from a P-R interval of 0.18 second up to 0.25 second on the tenth day. From a normal level of 0.25 mm. per minute the corrected sedimentation index rose to 1.60 mm. per minute. Hemolytic streptococci were present in the throat cultures before the protein shock, but absent afterwards. We have considered this a definite recrudescence of rheumatic fever following protein shock.

### Case IV

A. C., H.G.S. 5351, male, age 10, gave a history of four previous attacks of rheumatic fever and one of chorea during the preceding five years, in spite of which he had developed only minimal rheumatic heart disease, with the murmurs of slight mitral disease together with slight cardiac enlargement. In March, 1933, he again developed rheumatic fever, precipitated by a cold, with polyarthritis, nosebleeds, loss of weight and mild choreiform movements. On April 13, 1933, he entered the hospital for his second admission, where his subsiding joint pains as well as his fever responded promptly to salicylates.

*Observation I.* As shown in Figure 9, protein shock was induced by the intravenous injection of 0.1 cc. of typhoid-paratyphoid vaccine, which gave rise to a severe chill and a rather unusual temperature reaction to 105° F. (rectal). Within an hour from the onset of the chill two joints of the left hand, which had been involved on admission, became acutely tender, definitely red, hot and swollen. On the second day another finger and the wrist joint showed similar involvement. Because of the persistent and brisk temperature reaction, together with the considerable joint discomfort, salicylates were started with a prompt and striking subsidence of both fever and arthritis, even with minimal doses. Slight joint pains and an occasional slight fever persisted during the subsequent two weeks. It is of interest that the mild choreiform movements became quiescent after the febrile reaction. The corrected sedimentation index on April 23 was 1.40 mm. per minute. A study of the pharyngeal flora was not made during this episode. There were no further recrudescences, and by June, 1933, all clinical and laboratory evidence of infection had subsided. The heart remained as at entry. It is to be noted that clinical evidence of rheumatic fever was present in this patient at the time of the protein shock, and was manifested by recently swollen and painful joints which had been relieved by salicylates, by mildly active chorea and by a leukocytosis of 16,000.

**FEB. 1934**    **NAME E.F.**    **FEMALE**    **AGE 16**    **NO. 5676**

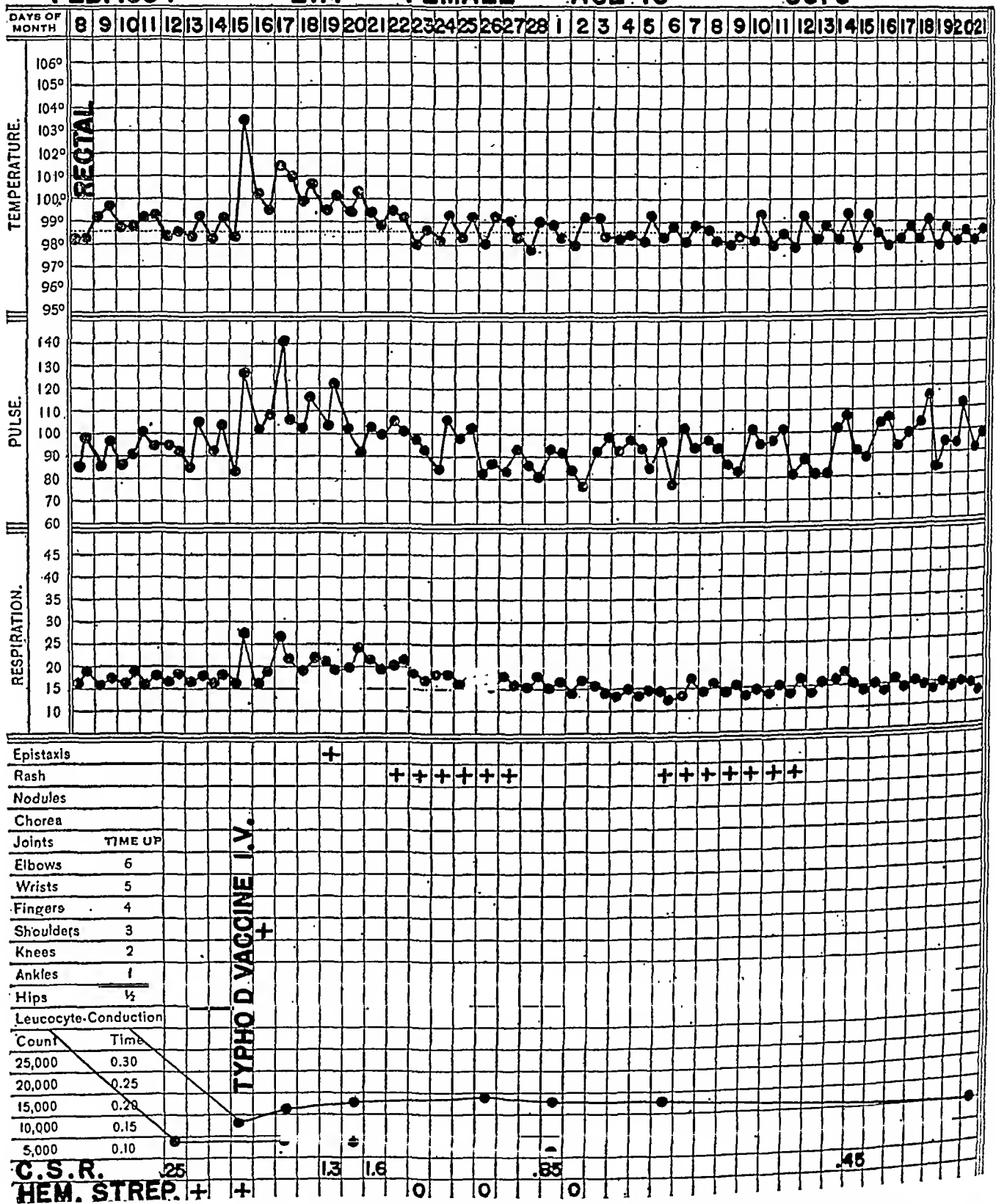
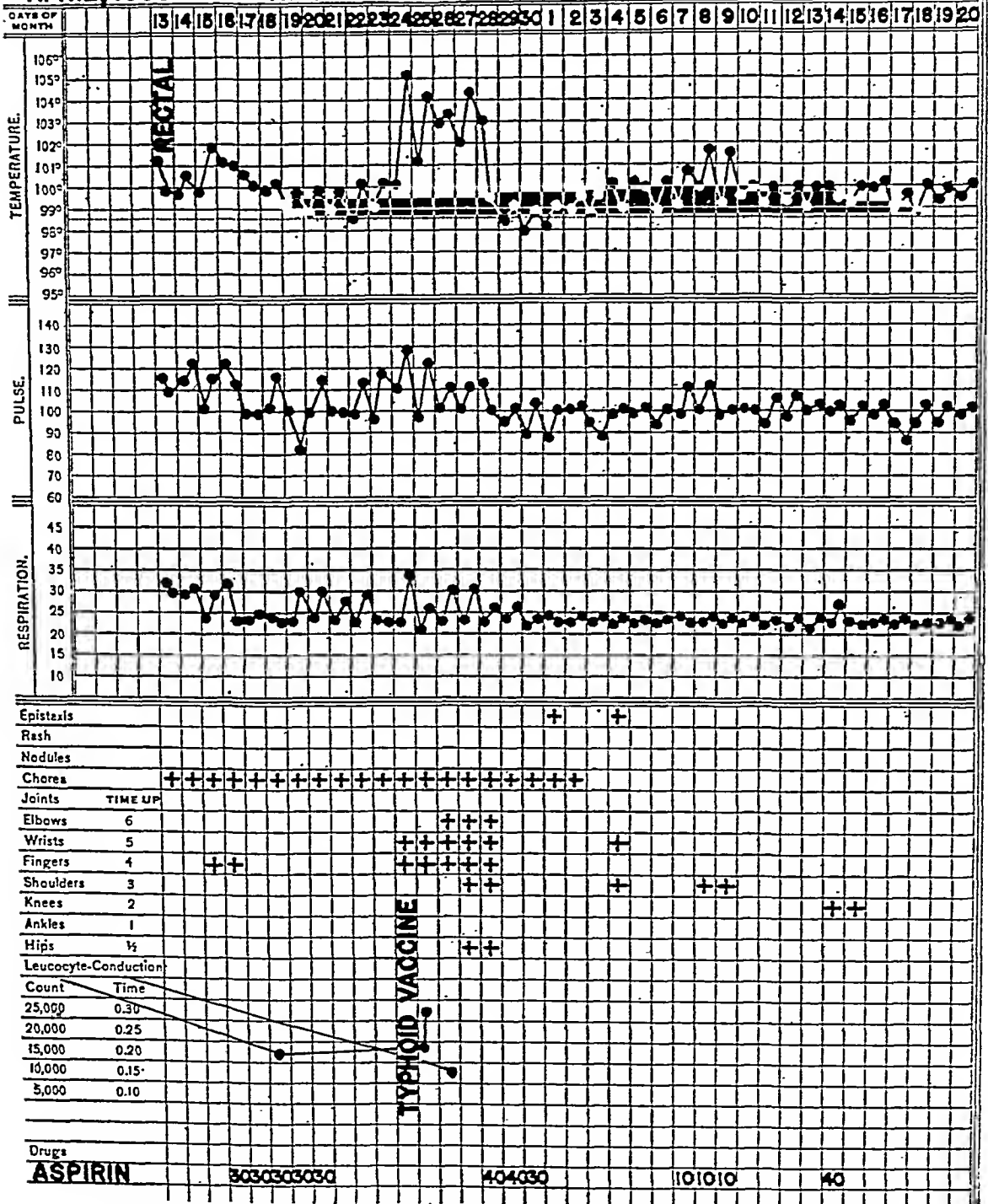


FIG. 8. AN APPARENT REACTIVATION OF RHEUMATIC FEVER FOLLOWING PROTEIN SHOCK.

FIG. 8. AN APPARENT REACTIVATION OF RHEUMATIC FEVER FOLLOWING PROTEIN SHOCK.

Of unusual interest here is the slightly delayed but progressive increase in the auriculoventricular conduction time by electrocardiogram from 0.18 second to 0.25 second.

APRIL, 1933 NAME A.C. MALE AGE 10 NO. 5351



## FURTHER OBSERVATIONS

In addition to, and concurrently with, the above recorded observations, five other patients of similar ages with either low-grade subclinical rheumatic fever or recently quiescent infection received a single protein shock by means of an intravenous injection of 0.1 cc. of typhoid-paratyphoid vaccine. The general reaction with chill and fever was similar in all respects, but subsequent clinical and laboratory study failed to reveal frank evidence of a reactivation of the rheumatic process. In two instances there was a short period in which doubtful signs and symptoms suggestive of rheumatic fever occurred. In one of the above instances a comparable fever produced by means of the hyperthermia box was not followed by any evidence of a recrudescence.

In summary, it may be stated that ten patients have received twelve injections of typhoid-paratyphoid vaccine. The usual chill and temperature were noted. In six instances clinical symptoms of active rheumatic fever appeared either immediately following the injection or after an interval of two to three weeks. In two instances there was a questionable reaction of this type. In the remaining four instances no clinical or laboratory evidence of a reactivation of rheumatic fever was demonstrable. No untoward effects of serious import were noted. Following the reactions to typhoid-paratyphoid vaccine there seemed to be a more rapid progress to quiescent rheumatic fever than was previously noted.

## DISCUSSION

Respiratory infection and its relation to rheumatic fever has for years received much attention. The recurrence of rheumatic fever following respiratory infection, often after an interval of a few days to two or three weeks, is generally known to students of the disease. It is also well recognized that recurrences are observed in the absence of evident previous infection or of other disturbing factors. In our series there has been a preceding respiratory infection in 75 per cent of observed recurrences; whereas, in the remaining 25 per cent there were no obvious precipitating causes. On the other hand it may be well to point out that attacks of definite tonsillitis or pharyngitis are not consistently followed by re-

currences of rheumatic fever. That other events, such as tonsillectomy, may at times be associated with subsequent rheumatic fever has also been noted in the past. Attention is called to a variety of episodes which appear to have been related to recurrent rheumatic fever. If one omits scarlet fever and other infections usually associated with infection of the upper respiratory tract, there still remain other events which appear to be of importance. Under these circumstances, a reliable history must be obtained soon after the event has transpired, as is often necessary even in the case of respiratory infections, since transient minor illness or injury may be quickly forgotten by the patient. Although it is obvious that the relative number of such events is small in so large a group of cases, no statistical study has been presented for two reasons: first, a history from this point of view is reliable only if one is cognizant of the possibility of such a relationship; and second, as noted above, it is necessary to obtain the history while events are fresh in the patient's mind. The majority of our patients have had rheumatic fever for a considerable period of time prior to admission to the hospital, and only recently has this sequence of events been searched for. Second, in a hospital such as ours traumatic accidents are not observed, nor are illnesses other than rheumatic fever common. A reliable statistical study, although difficult to obtain, we believe would be valuable. Since we have been aware of the possible importance of events other than streptococcal infections prior to recurrences of rheumatic fever, the frequency with which they have been encountered has increased surprisingly.

It is not rare to find instances in which an abdominal operation is a precursor of clinical rheumatic fever. This would seem to hold, regardless of the need for the surgical procedure or the diagnosis for which it is undertaken.

It seems evident to us that it is not rare for events other than respiratory infection to precede the appearance of clinical rheumatic fever, and that they must be considered in some manner a precipitating factor, possibly non-specific.

The clinical observation of the reappearance of the symptoms of rheumatic fever following a single treatment with non-specific protein therapy



seems of considerable importance to us. Rheumatic fever was not produced in normal human beings, but the asymptomatic phase of the disease was brought to a clinical level in known rheumatic individuals by non-specific means. The frequency and definiteness of these few observations convince us that we are dealing with more than a chance relationship. The studies have not been pursued further for the obvious reason that we did not wish to cause clinical disturbances in our patients. It is generally accepted that subjects with rheumatic fever, especially those with recent histories of rheumatic fever, are very susceptible to recurrences. It is evident that these may be precipitated frequently by purely non-specific means. These observed recurrences were, on the whole, of shorter duration and were milder than those occurring naturally. No evident alteration of the cardiac state, other than the temporary prolongation of the auriculoventricular conduction time, was noted. The symptoms noted during the recurrences of clinically active rheumatic fever, following the reaction to a single injection of non-specific protein, reproduced in a striking fashion the rheumatic manifestations which had been previously observed in the individual patient during a naturally occurring recrudescence.

As noted previously, the initiating factor most commonly encountered in patients with rheumatic fever is respiratory infection. In view of the data presented, bacterial activity during respiratory infection may prove to be of secondary importance. The crowded, unhygienic living conditions of the majority of patients with rheumatic fever may well account for the frequency of respiratory infections. In addition, the study of any group of patients with rheumatic fever will show a large number of such respiratory infections even under satisfactory living conditions.

In view of the observations herein recorded, it is suggested that the rôle of infections or of episodes seemingly related to recurrent rheumatic fever may not be as specific as has been previously thought. Respiratory infection, often accompanied by hemolytic streptococci, is frequently the precipitating agent of rheumatic fever, but other events likewise may be important. The appearance of the clinical symptoms and signs of the disease are not, therefore, entirely dependent

upon recognizable respiratory infection. This apparently non-specific relationship may be of importance in future etiological considerations.

### CONCLUSIONS

1. There appears to be no significant clinical difference between the recurrences or recrudescences of rheumatic fever following (1) respiratory infection, (2) other forms of infection, (3) accidents or operative procedures, and (4) a single intravenous injection of typhoid-paratyphoid vaccine sufficient to cause a slight febrile reaction and chill.

2. The probable significance of these observations has been discussed. It is evident that various events precede and apparently influence the appearance of the signs and symptoms of recurrent rheumatic fever.

3. It seems desirable, in view of the observations presented, to consider the rôle of such events as non-specific until more definite information is available concerning the etiological agent.

We wish to gratefully acknowledge our indebtedness to Doctors James H. Means and M. N. Smith-Petersen of the Massachusetts General Hospital for allowing us to cite cases from their respective services. The typhoid-paratyphoid vaccine used throughout these observations was obtained from the Antitoxin Laboratory of the Massachusetts Department of Public Health.

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# FURTHER OBSERVATIONS ON THE LEUKOCYTIC RESPONSE INDUCED BY THE INTRAMUSCULAR INJECTION OF LIVER EXTRACT<sup>1</sup>

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(Received for publication May 28, 1935)

The leukocytogenic effect of the intramuscular administration of liver extract (Lederle) to normal individuals has been presented in a previous paper (1). The response in every instance was due both to an actual and relative increase in the polymorphonuclear neutrophils. The purpose of the present communication is two-fold: first, to submit suggestions regarding the source of this leukocytic response; and secondly, to determine the value of its clinical application in the treatment of surgical patients with infection and no leukocytosis, or definite leukopenia.

## 1. Source of the leukocytic response

*Method.* The subjects were all normal individuals, either members of the staff, secretaries, and technicians, or convalescent, afebrile, surgical patients on the wards of the Mary Imogene Bassett Hospital.

Two methods of experimental procedure were followed. In the first group, a series of observations covering 3 consecutive days was carried out according to the method of Powers and Murphy (1). The first and third days were utilized as control periods in order to obtain the normal daily range of the leukocytic level and the average differential Schilling count at representative hours before and after the administration of liver extract. At 9 o'clock on the morning of the second day an additional control count was taken and directly thereafter 3 cc. of liver extract<sup>2</sup> were injected intramuscularly in the gluteal region. Complete studies were then made hourly until 7 o'clock in the evening. Thus it was possible not

only to confirm the previous experimental results (1) but also to determine whether the attendant leukocytosis was due to the liberation of mature neutrophils, juvenile forms, or both.

The second group of observations covered a period of 18 consecutive hours. A control count was taken at 5 o'clock in the morning and 3 cc. of liver extract were injected immediately thereafter. Complete determinations were then made at hourly intervals until 11 o'clock in the evening. It was hoped thereby to obtain more information concerning the duration of the leukocytic response and to gather some data regarding the maturation of the immature cells, should these be found to be increased in the peripheral circulation.

Blood for each count was taken from the finger into a standardized pipette, diluted, shaken for 3 minutes and counted in a standardized Levy counting chamber. Both pipettes and hemocytometers were certified by the U. S. Bureau of Standards and the same instruments were used for all the observations on any one subject. Duplicate cover-slip preparations were made in the usual manner and stained with Wright's stain. Each smear was counted separately, and at random, in order that the result might not be affected by memory of the previous differential identification for the same hour.

One hundred cells in each of the 2 preparations were classified according to the method of Schilling. In general the results varied within a narrow range; the average was utilized in preparing the charts.

*Results.* There were 4 individuals in the first group. The results are presented in composite, graphic form in Figure 1. The curves representing the total white count, the total number and percentage of polymorphonuclear neutrophils are identical with those presented in the previous paper and require no discussion, except to point

<sup>1</sup> Read before the American Society for Clinical Investigation, Atlantic City, May 6, 1935.

<sup>2</sup> This extract was prepared by the Lederle Laboratories, Inc., and furnished through the kindness of Dr. Guy W. Clark.

out again that the leukocytic response is entirely neutrophilic in character. The point of major importance in the present studies is the relatively greater increase in both total number and percent-

age of young forms compared with the mature cells. Reference to Table I discloses the fact that while the total number of neutrophils per cu. mm. increased 112 per cent, the band forms, or young cells, increased 235 per cent while the segmented forms gained only 96 per cent. The number of

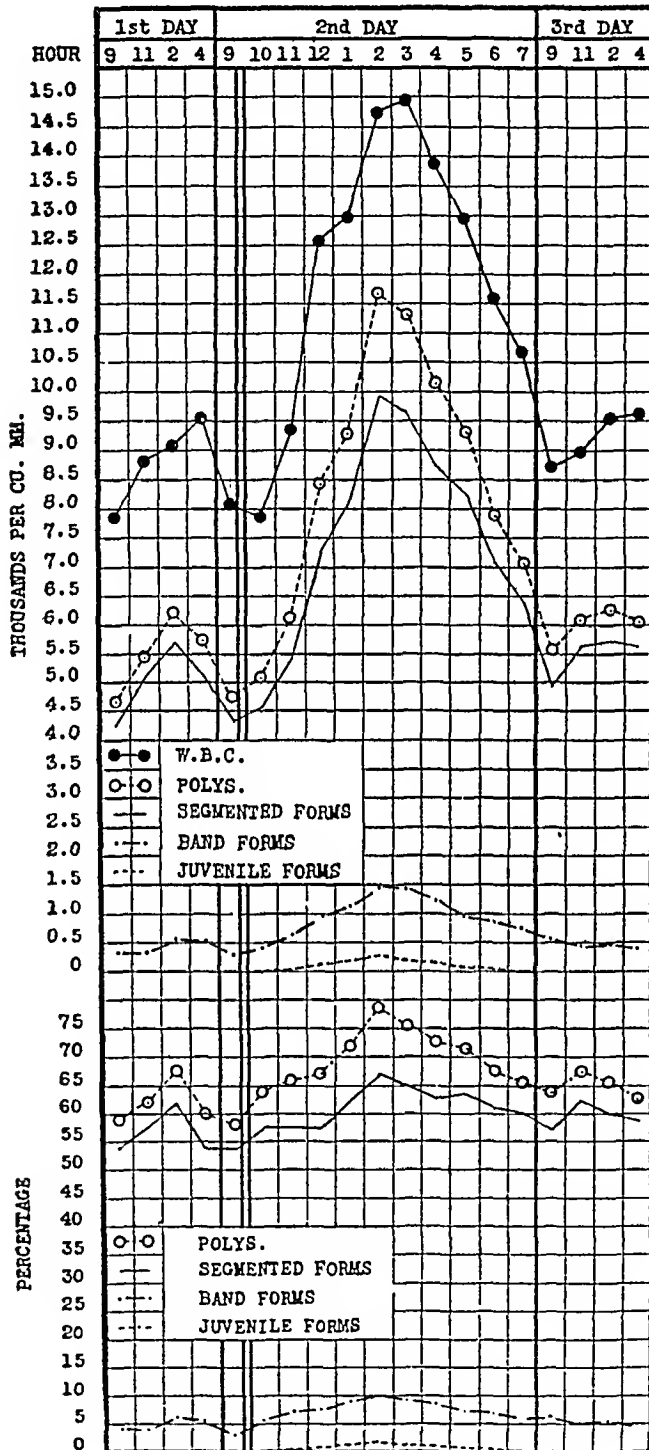


FIG. 1. A COMPOSITE GRAPH OF THE TOTAL WHITE CELLS PER CU. MM. AND THE TOTAL NUMBER AND PERCENTAGE OF NEUTROPHILS, SEGMENTED FORMS, BAND CELLS AND JUVENILES OF 4 NORMAL SUBJECTS.

The single heavy vertical lines separate the chart into days; the intramuscular injection of 3 cc. of liver extract is indicated by the double line.

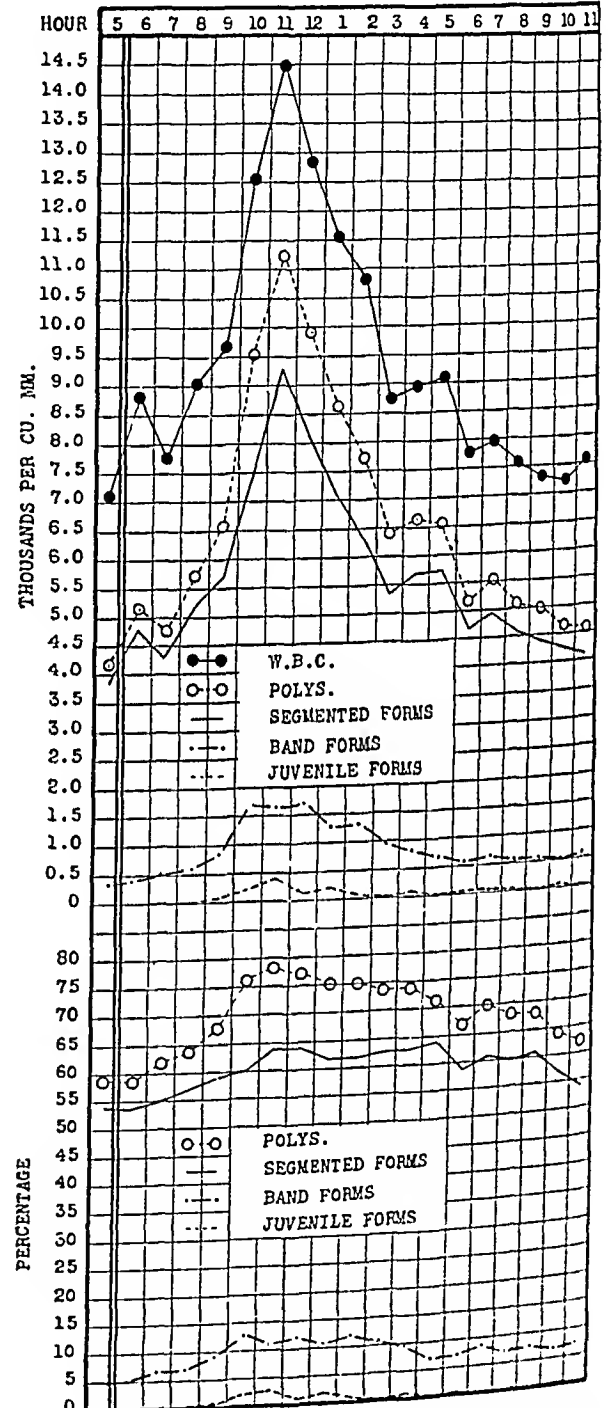


FIG. 2. A COMPOSITE GRAPH OF THE TOTAL WHITE CELLS PER CU. MM. AND THE TOTAL NUMBER AND PERCENTAGE OF NEUTROPHILS, SEGMENTED, BAND AND JUVENILE FORMS OF 3 NORMAL SUBJECTS DURING 18 CONSECUTIVE HOURS AFTER THE INTRAMUSCULAR INJECTION OF LIVER EXTRACT.

TABLE I

*Tabulation of the actual increase per cu. mm. and the percentage increase of total white cells, polymorphonuclear neutrophils, segmented, band, and juvenile forms at the peak of the leukocytic rise in the first series of experiments*

	W.B.C.	Total polymorpho-nuclears		Segmented forms		Band forms		Juvenile forms	
	per cu. mm.	per cu. mm.	per cent	per cu. mm.	per cent	per cu. mm.	per cent	per cu. mm.	per cent
Average of control observations.....	8,850	5,520	62	5060	57	440	4.9	0	0
Peak of rise.....	14,770	11,670	79	9900	67	1480	10.0	270	1.8
Actual increase.....	5,920	6,150	17	4840	10	1040	5.1	270	1.8
Percentage increase.....	67	112	27	96	17	235	104.0		

juveniles increased from zero to 270 per cu. mm. Expressed in terms of the Schilling differential formula the per cent of band forms more than doubled while that of the mature cells increased only 17 per cent.

The same facts are even more apparent in the second series of observations. This group was composed of 3 normal subjects to whom liver extract was administered immediately after blood was taken for control studies at 5 o'clock in the morning. A composite curve of the results is presented in Figure 2. As usual, the peak of the rise occurred 6 hours after the injection. The total number of white cells per cu. mm. doubled, and the neutrophils increased 169 per cent; while the number of mature cells multiplied 143 per cent, the band forms nearly tripled that figure by a maximum gain of 370 per cent. The juveniles increased from zero to 400 per cu. mm. (Table II). Expressed in terms of the Schilling differential again, the percentage of band forms increased 131 per cent while that of the mature cells gained only 18 per cent. In other words, a distinct shift to the left occurred in each series of experiments.

The longer period of observations in the second group possibly threw some light upon the phe-

nomenon of cellular maturation. The number of young cells in the peripheral blood decreased more slowly following the peak of the rise than did either the total neutrophils or the mature forms (Figure 2). From these experiments one might surmise that the transitional period of immaturity, during which the young cell passes from a myelocyte to an adult form with a multilobed nucleus requires a few hours only. The duration of the leukocytic response was essentially the same as in all previous experiments.

*Discussion.* These findings confirm the previous observations by the author (1) and also by Meyer, Middleton, and Thewlis (2) that the leukocytosis induced by the intramuscular injection of liver extract is entirely neutrophilic in character. Furthermore, they demonstrate that this leukocytic response is due to an increase in both young and mature cells in the circulating blood, with the increase in young forms proportionately greater, both numerically and relatively, than that of the adult cells. These observations suggest that the response is the result of some direct action or effect of liver extract upon the bone marrow. Meyer and his collaborators (2) came to this same conclusion although they were unable to demonstrate a shift to the left in the Schilling counts of

TABLE II

*Tabulation of the actual increase per cu. mm. and the percentage increase of total white cells, neutrophils, segmented, band, and juvenile forms at the peak of the rise in the second group of experiments*

	W.B.C.	Total polymorpho-nuclears		Segmented forms		Band forms		Juvenile forms	
	per cu. mm.	per cu. mm.	per cent	per cu. mm.	per cent	per cu. mm.	per cent	per cu. mm.	per cent
Average of control observations.....	7,080	4,180	59	3820	54	340	4.8	0	0
Peak of rise.....	14,440	11,260	78	9280	64	1600	11.1	400	2.8
Actual increase.....	7,360	7,080	19	5460	10	1260	6.3	400	2.8
Percentage increase.....	104	169	32	143	18	370	131.0		

4 normal individuals at the peak of the rise. Such a shift did occur, however, in 1 patient with Banti's disease following the intramuscular injection of liver extract (Lederle). In this patient, and in 1 other with splenomegaly, well marked leukocytic responses followed the parenteral use of extract, both before and after removal of the spleen. In fact, the leukocytosis was more marked in each instance after splenectomy than before. This rise was preceded by a drop in the leukocytic level immediately following the injection, an observation which is in direct opposition to the studies of Miller and Rhoads (3).

These observers describe an increase in all the formed elements of the blood, including the leukocytes, 20 minutes after the intravenous administration of liver extract to a patient with anemia and splenomegaly. This response was coincidental with contraction of the spleen which could be demonstrated both clinically and roentgenologically, and they conclude that the spleen must be considered not only as a reservoir of red blood cells but also as a storehouse for proportionately greater numbers of white cells and blood platelets. Similar splenic contractions were induced by the intramuscular administration of adrenaline, histamine, and eserine. Injection of the 2 former drugs was followed promptly by an increase in the number of leukocytes in the peripheral blood; no reaction was noted after the use of eserine. These observations are interesting in view of the marked leukocytosis which followed the injection of extract *after* splenectomy in the patients of Meyer and his collaborators. One must conclude from their experiments at least that the spleen did not act as a reservoir for the white cells, and that the leukocytosis which followed the intramuscular injection of liver extract was due either to direct or indirect stimulation of the bone marrow.

## 2. Value of liver extract in the treatment of certain types of infection

In the large majority of cases the presence of pathogenic organisms in the human body is, in itself, a powerful leukocytogenic stimulant. There are certain patients, however, either so overwhelmed by an acute process, or so debilitated by chronic infection that no leukocytic response takes

place. It was in these groups of individuals that the practical value of liver extract was tested in a very small series of cases. Only 3 patients thus far have seemed suitable for such a clinical trial.

### PROTOCOLS

#### Case 1

The patient was a married woman, 26 years of age, who was referred to the hospital because of chills and a high fever. A spontaneous abortion occurred 1 month previously. Persistent vaginal bleeding terminated in a severe hemorrhage 2 weeks later. The vagina was packed by her family doctor; severe abdominal cramps ensued, and the packing was dislodged by a second hemorrhage. Except for headache and a slight fever she was then free from symptoms until the day before admission when she had a sudden chill with elevation in temperature to 104° F.

The patient was exceedingly toxic and presented the appearance of an individual with an overwhelming infection. Except for flushed cheeks, the skin and mucous membranes were pale. There was no abdominal tenderness or spasm. Motion of the cervix was painful; the uterus was enlarged, boggy, and tender.

The temperature was 104° F., and the pulse rate was 134; the following day the temperature rose to 105.6° and the pulse to 140. Blood cultures failed to substantiate the clinical impression of bacteremia. The patient ran a spiking fever with wide daily swings ranging between 98.6° and 105.2° for 2 weeks. Thereafter the temperature gradually subsided to normal and she was discharged 26 days after admission.

*Diagnoses:* Early abortion, incomplete; endometritis, septic; anemia secondary to acute and chronic loss of blood.

*Procedure.* When these observations were made, the usual interval between the injection of extract and the maximal leukocytic response in normal individuals had not been determined. Hence 10 o'clock in the morning and 4 in the afternoon were chosen as arbitrary hours for acquiring samples of blood. Injections of 3 cc. of liver extract, indicated by the single vertical lines in Figure 3, were made at 8 o'clock in the evening of the preceding day as represented on the chart. Direct transfusions of 500 cc. of whole blood, shown by the double vertical lines, were given on the second and eighth days in the hospital.

*Result.* In spite of the first transfusion the white count dropped precipitously during the subsequent 3 days from 18,050 to 8,900 and the neutrophils from 13,740 to 6,320. After the first and second injections of liver extract an even more abrupt rise occurred; the leukocytes jumped from 10,000 to 20,900 and the neutrophils from 7,500 to 17,970 per cu. mm. The response after the third administration is difficult to evaluate because of the subsequent transfusion which was warranted by the extreme seriousness of the patient's condition. Two later injec-

tions were each followed by a definite but less spectacular elevation of the leukocytic count.

The detailed observations are presented graphically in Figure 3 and are correlated with the temperature, erythrocytic count, and percentage of hemoglobin in Figure 4.

### Case 2

The patient was a 33 year old housewife who induced an abortion by the introduction of a catheter 8 days prior

to admission to the hospital. The following morning she passed a 2 months' fetus, placenta and intact membranes. After 4 days of moderate vaginal bleeding, the discharge became foul; fever, headache, and general malaise ensued.

The patient was extremely pale except for a malar flush. The skin and mucous membranes were dry and the tongue was coated. The abdominal wall was flabby but soft throughout and free from tenderness except in the suprapubic region; with deep inspiration the spleen

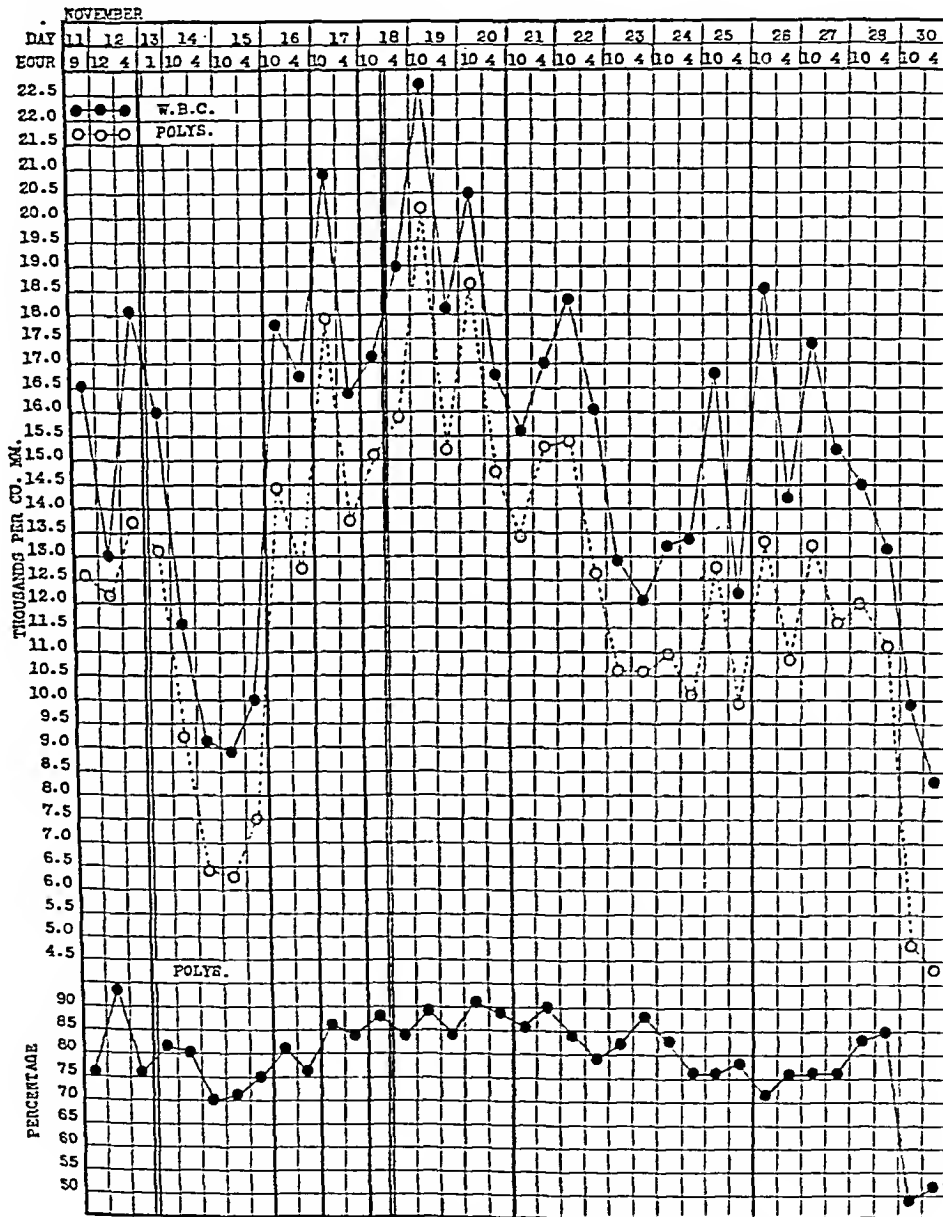


FIG. 3. CHART OF THE INDIVIDUAL OBSERVATIONS IN CASE 1.

The injections of extract are indicated by the single heavy vertical lines, transfusions of whole blood by the double lines.

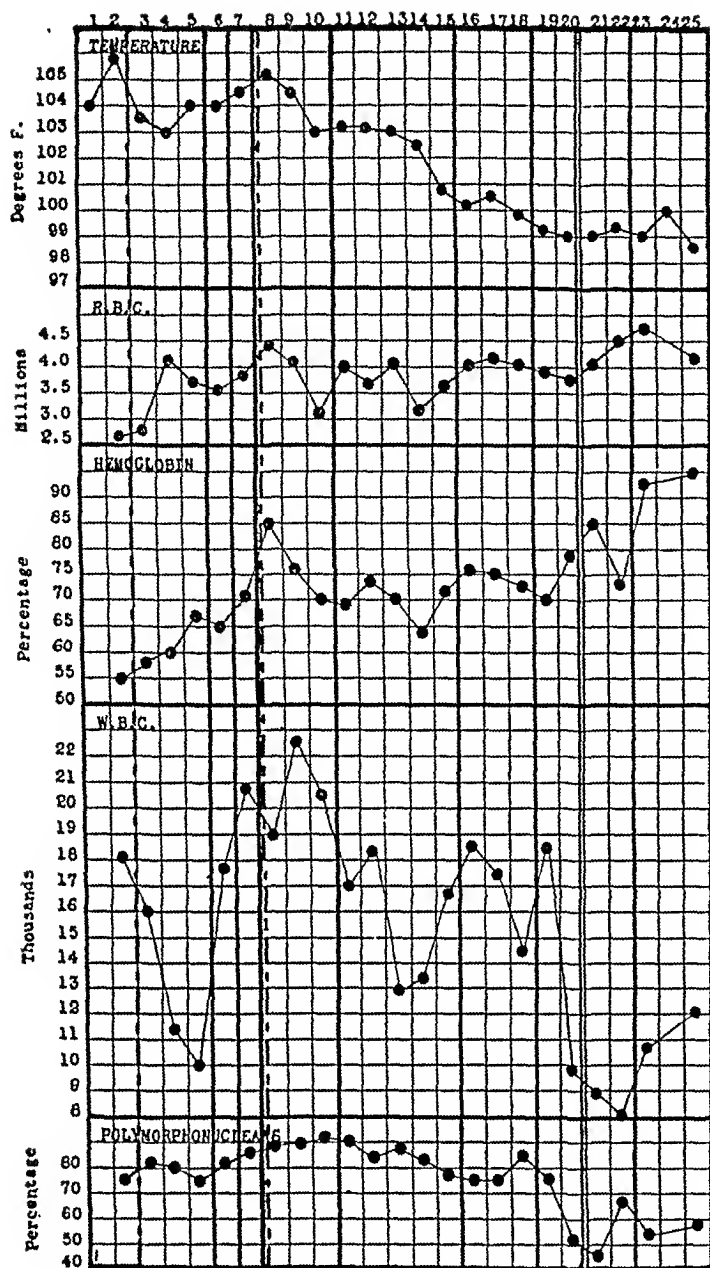


FIG. 4. CORRELATION OF THE HIGHEST DAILY TEMPERATURE, ERYTHROCYTIC COUNT, HEMOGLOBIN, LEUKOCYTIC COUNT AND PERCENTAGE OF NEUTROPHILS IN CASE 1.

The broken vertical lines indicate transfusions of whole blood, the single solid vertical lines represent intramuscular injections of liver extract, and the double solid vertical lines indicate the beginning of daily oral administration of ferric ammonium citrate.

was palpable 2 cm. below the costal margin. The cervix was soft and patulous, the uterus was enlarged and tender to bimanual palpation; induration and tenderness were present in the posterior cul-de-sac.

The temperature on admission was 103.4° F., the pulse rate was 110, the erythrocytic count was 2,230,000, the hemoglobin 48 per cent by the method of Sahli; the leukocytes were only 3,380 per cu. mm. with 75 per cent polymorphonuclear neutrophils. The subsequent white counts are presented graphically in Figure 5. Culture

of the vaginal discharge yielded *Streptococcus anhemolyticus* and *Escherichia coli*.

With conservative treatment the temperature and pulse rate subsided to normal in 3 days. The vaginal discharge gradually ceased and the patient was allowed to go home 1 week later. The erythrocytic count at that time was 3,100,000 and the hemoglobin was 55 per cent.

**Diagnoses:** Early abortion, complete; endometritis, septic, due to *Streptococcus anhemolyticus* and *Escherichia coli*; anemia secondary to chronic loss of blood.

**Procedure.** The experiments with normal individuals had been conducted prior to the treatment of this patient. Hence a method as nearly comparable as possible was carried out. Three cc. of liver extract were given intravenously at 9 o'clock in the morning of the second, third, fourth, sixth, ninth, and tenth days. Blood for counts and smears was taken each afternoon at approximately two-hourly intervals.

**Result.** The result is charted graphically in Figure 5. The patient had a pronounced leukopenia on admission, with only 3,380 leukocytes and 2,060 neutrophils, per cu. mm. An immediate response occurred after each injection, with the peak of the rise at about the same interval of time as was noted with normal individuals. A gradual but definite upward trend in the average leukocytic level from day to day also took place, until a maximum of 12,120 white cells and 9,210 neutrophils was reached. The patient's convalescence was uneventful.

### Case 3

The patient, a married woman, 24 years of age, was admitted to the hospital with puerperal sepsis, 8 days after delivery.

Examination disclosed a pale, prostrated, acutely ill individual with dry, parched lips and a coated tongue. The neck was stiff and painful when flexed. The heart was hyperactive, the impulse forceful, and the rate rapid. The abdomen was enormously distended without spasm and with no tenderness except in the suprapubic region. After the abdominal distension had partially subsided, the uterine fundus could be felt mid-way between the symphysis and umbilicus.

The temperature was 104° F. and the pulse rate, 136. The white count was 12,750 with 80 per cent neutrophils, the red count, 2,990,000, and the hemoglobin 60 per cent. The leukocytic counts in detail and their relations to injections of liver extract, transfusions, and the administration of antistreptococcus serum (New York State Department of Health) are presented in Figure 6.

Vaginal smear yielded *Streptococcus hemolyticus* and the same organism was obtained from the blood on the seventh day and on numerous occasions thereafter.

During the first week in the hospital clinical improvement and a downward trend in the temperature were noticeable. She was transfused on the sixth day. On the seventh, the temperature was again over 104° F., and the course thereafter was exceedingly septic. Thrombophlebitis of the left iliac vein developed and death occurred at the end of 5 weeks.

**Diagnosis:** Puerperal septicemia and bacteremia due to *Streptococcus hemolyticus*; acute thrombophlebitis of the vessels of the left lower extremity; anemia secondary to infection.

**Procedure.** The first 36 hours were utilized as a control period; blood for the usual studies was taken at

with a peak of 20,000 nine hours after the last injection (Figure 6). An abrupt drop occurred the following day in spite of a transfusion. The trend thereafter was steadily downward to a constant average level of about 10,000 per cu. mm. which was maintained during the remainder of the patient's illness.

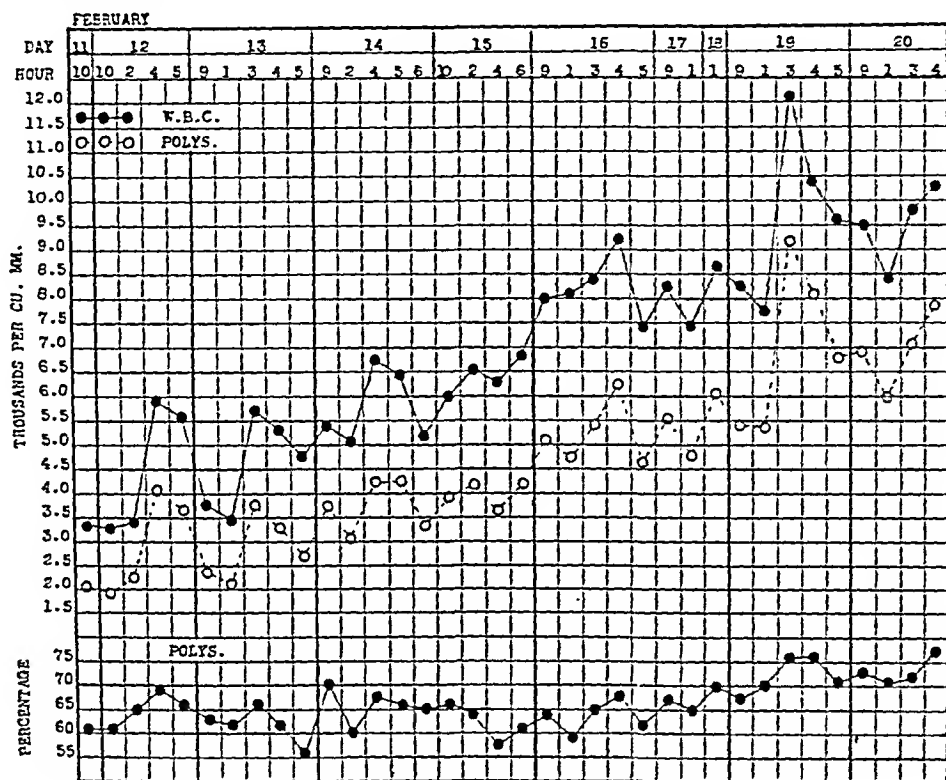


FIG. 5. CHART OF THE INDIVIDUAL OBSERVATIONS IN CASE 2.

The injections of liver extract are indicated by the single vertical lines.

intervals of 2 hours during the day in order to obtain the range of the leukocytic level before treatment was instituted. The patient was then given 3 cc. of liver extract intramuscularly at 8 o'clock in the morning of the following 3 successive days and the observations were repeated at the same two-hourly intervals. The author was not personally in charge of this patient and the injections were discontinued thereafter. Blood for counts was taken at less frequent intervals for several days longer, however, and the leukocytic curve presents a sharp contrast to that which was observed during the period of treatment with liver extract.

**Result.** During the first 6 days in the hospital the patient's clinical condition improved. The highest daily elevation in temperature dropped from 104° F. to 102° F. and the pulse rate showed a perceptible downward trend. Three injections of liver extract were given during this period and the total white count rose from an average level of 10,390 per cu. mm. on the day before treatment was instituted to an average of 17,230 on the third day

**Comment.** No conclusions may be drawn from such a small group of patients. In each of the cases presented, however, a well marked elevation of the leukocytic count followed the intramuscular use of liver extract. Two patients, who were exceedingly ill upon admission to the hospital, recovered but it is very probable that recovery would have occurred without the use of extract as a leukocytogenic stimulant. In one case, the eventual outcome was fatal but clinical improvement was apparent during the period of treatment.

Several favorable reports (4), (5) have appeared in the recent literature on the value of liver extract in the treatment of agranulocytic angina. Murphy (6) has recorded well marked leukocytic responses with recovery in several cases, and feels that the drug has a distinct place in the treatment



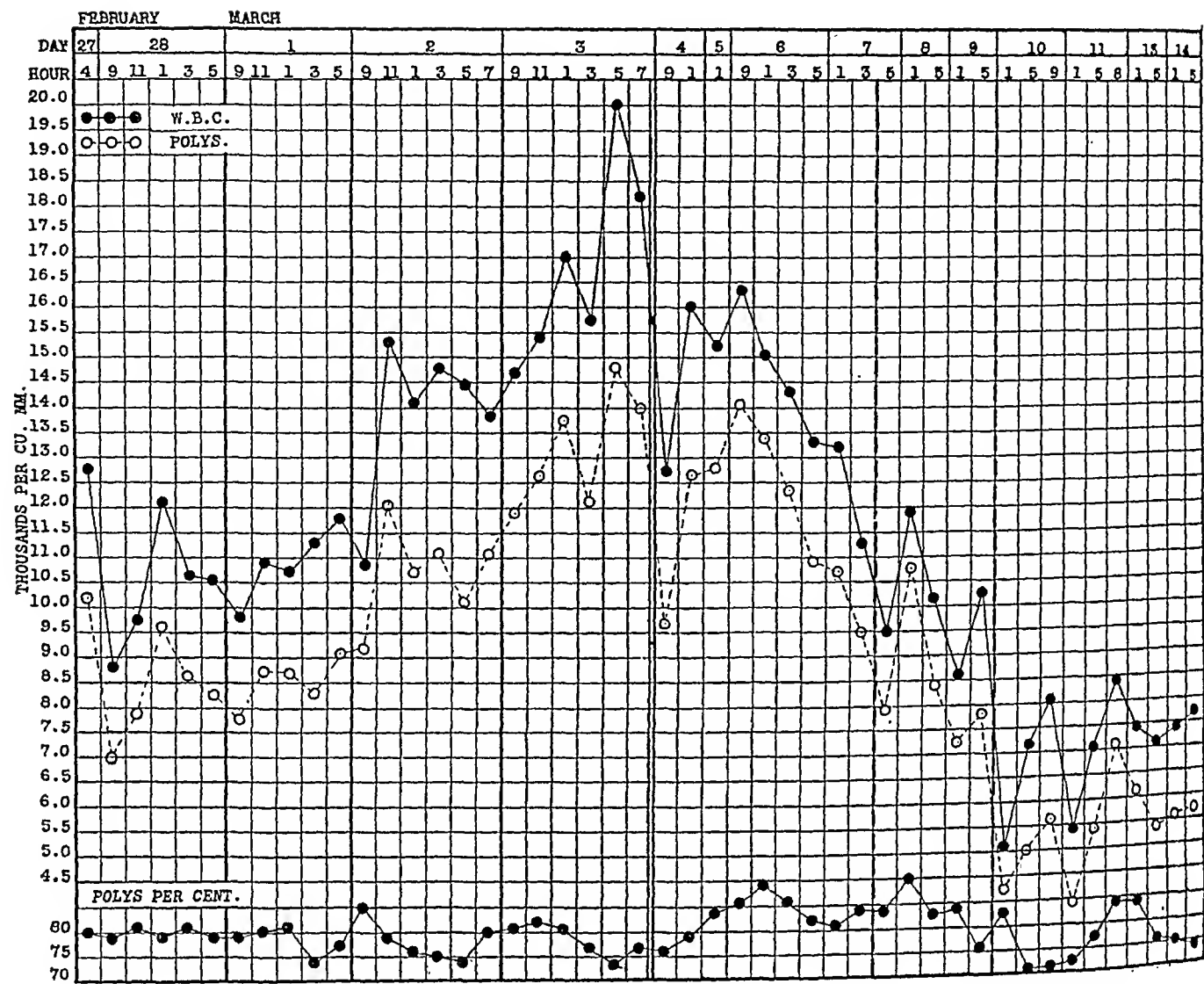


FIG. 6. CHART OF THE INDIVIDUAL OBSERVATIONS IN CASE 3.

Injections of liver extract are indicated by the single vertical lines, a transfusion of blood by the double lines, and the administration of antistreptococcus serum by the broken lines.

of this disease. If such be true, it seems reasonable to expect that some benefit may be derived from its use in patients with infection accompanied by a less severe degree of leukopenia than that which occurs in the average case of agranulocytosis.

SUMMARY

Observations on the degree and character of the leukocytic response induced by the intramuscular administration of liver extract to 7 normal subjects and to 3 surgical patients with infection have been described and presented in graphic form.

The normal individuals were divided into 2 groups. To the first of these 3 cc. of liver extract were given at 9 o'clock in the morning; ob-

servations made the day preceding and the day following the experiment were utilized as controls. The leukocytic response was entirely neutrophilic in character, and reached its peak in 6 hours, and was composed of a relatively greater increase (235 per cent) in the young or band forms than in the mature cells (96 per cent). The number of juveniles increased from zero to 270 per cu. mm.

There were 3 normal subjects in the second group who received the same amount of extract at 5 o'clock in the morning. The total white cells doubled, the neutrophils increased 169 per cent and the band forms, 370 per cent. The juveniles rose from zero to 400 per cu. mm. The peak of the rise again occurred 6 hours after the injection. The number of young cells disappeared relatively more slowly from the peripheral blood than did

the mature forms. The results suggest that the leukocytosis induced by the intramuscular administration of liver extract to normal individuals is due either to direct or indirect stimulation of the bone marrow.

Three surgical patients, 1 with puerperal sepsis and bacteremia, and 2 with septic endometritis were given liver extract intramuscularly. In each case the leukocytic count was low before the injections were started and a well marked leukocytosis occurred thereafter. The response was entirely neutrophilic in character.

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# THE RELATIONSHIP BETWEEN INSENSIBLE WATER LOSS AND HEAT PRODUCTION IN PATIENTS WITH HYPOTHYROIDISM COMPARED WITH NORMAL SUBJECTS<sup>1</sup>

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In normal subjects in the basal state the heat lost from the body through evaporation of water from the skin and respiratory passages has been found to be related to the total heat production. Soderstrom and DuBois (1) in 1917 measured the basal heat production and the water of vaporization from the body simultaneously in a Russell-Sage calorimeter; 27 studies in 12 normal male subjects, 20 to 50 years of age, revealed that an average of 24 per cent, with variations from 21 to 28 per cent, of their total heat production was expended in the vaporization of water. Studies in 8 boys and 6 old men showed an average of 27 per cent of the total heat lost through vaporization in each group (1). Subsequently, Levine and Wilson (2) found that infants and children lost in this manner an average of 26 per cent of their heat production. Benedict and Root (3) in 1926 measured the basal metabolic rate and either the basal or sleeping insensible water loss in a large group of individuals comprising normal subjects and patients with diabetes or thyrotoxicosis; from their data these authors constructed a table for predicting metabolism from the basal or sleeping insensible weight loss. The studies of Benedict and Root (3) and those of subsequent investigators (4, 5, 6, and others) confirm in general those of Soderstrom and DuBois in normal subjects. On the basis of data available in the literature (1, 2, 6) Lavietes (7) has recently described an average relationship between basal insensible loss of weight and basal heat production in normal subjects by the equation: Calories (per hour) =  $2.2 I L$ , where  $I L$  represents the insensible weight loss in grams per hour.

Attempts to correlate insensible water loss with metabolism under conditions other than basal have yielded varying results. It has been found that

the insensible weight loss increases above the basal when a patient assumes the sitting position (8), on activity (9, 10), in fever (1), and usually after eating (1, 11, 12). Jores (11) and Dieckhoff (12), however, found that the increase in metabolism following a protein meal was not always associated with a corresponding increase in insensible water loss, in fact in certain experiments the insensible loss did not increase at all when the metabolism increased by as much as 30 per cent. Furthermore, results of other investigators have revealed that the heat lost by vaporization in sleep (8, 9, 13) and on activity (7), even when unaccompanied by sensible perspiration, may not bear the same relation to total heat production as obtains under basal conditions.

To what extent the percentage of the total heat lost by water vaporization varies in metabolic diseases which alter heat production is not entirely clear. Thus, although the percentage of heat lost by vaporization in hyperthyroidism has been studied by several authors, studies in only a few isolated cases of hypothyroidism are reported. The percentage of the total heat lost through vaporization under basal conditions has often been found increased in hyperthyroidism (1, 3, 4, 11), even when no sensible perspiration is discernible; the few available reports in hypothyroidism indicate that the percentage heat lost through vaporization may be less than normal (1, 4, 9). The purpose of the present investigation was to determine the relationship of insensible water loss to heat production in a group of patients with hypothyroidism. By comparing the values in hypothyroidism with values in normal individuals under identical experimental conditions, certain differences became evident.

## MATERIAL AND METHODS

Nine studies were made in eight patients with hypothyroidism which developed following total ablation of

<sup>1</sup> This investigation was aided by a grant from the William W. Wellington Research Fund of Harvard University.

the thyroid gland, performed for the relief of either angina pectoris or congestive heart failure (14). Some clinical signs and symptoms of hypothyroidism were present in all these patients at the time of study. In accord with considerations outlined elsewhere (14), thyroid (Armour's) medication was being administered to three of the patients in amounts sufficient to prevent distressing symptoms of myxedema (Table I). A few patients were studied just before thyroid therapy was started, when striking clinical manifestations of myxedema were present. Two additional studies were made in two of the patients with hypothyroidism after the metabolic rate had been increased by the administration of dinitrophenol for several days before the test. The results in the patients with hypothyroidism are compared with those obtained in eleven studies in nine individuals with no evidence of endocrine disease; seven of these tests were made in five individuals who were entirely normal; the diagnoses in the other four patients are given in Table I.

The experiments were done either after the patient had fasted over night or three hours after he had eaten a light breakfast (200 cc. of orange juice and a slice of toast with butter), consisting mainly of carbohydrate. After coming to the laboratory he removed his clothes and put on a hospital Johnny, outing flannel pants and cotton socks, thus covering the whole body except the hands and face. These clothes were hung in the laboratory for several hours and were weighed just before they were donned. The laboratory temperature was adjusted and controlled between 73° and 78° F.

A Troemner<sup>2</sup> "human being balance" with suspended seat was utilized for weighings. This balance in our hands appeared accurate to approximately 1 gram; although the weight measurements are not as exact as those obtainable with a more sensitive balance, they serve for the purpose of this investigation. The subject sat in the balance seat for at least 30 minutes, after which a metabolic rate measurement was made, the expired gases being collected for ten minutes in a Tissot spirometer. The weight of the patient was then taken, the time being noted exactly. The patient remained quiet on the balance during the following hour, after which another weight measurement was made. The metabolic rate measurement was then immediately repeated. Following this the patient stepped directly from the balance to a bed where measurements of the skin temperature at twenty-five points on the body, including points on the head, trunk, and both left and right extremities, were made with a standard thermocouple. The clothes were removed and immediately reweighed; in almost every instance the weight increased from 0 to 3 grams, the average gain being approximately 2 grams. Although there was an appreciable gain in the weight of the clothes as thus measured it is probable that equilibrium was obtained with the cutaneous water loss and the atmosphere during the hour which elapsed between dress-

ing and the beginning of the weighings to determine the insensible water loss, the change in the weight of the clothes during the hour when the insensible weight loss was measured probably being insignificant (10).

The average deviation from the average of the two metabolic measurements made in each experiment was  $\pm 2$  per cent, the range being from  $\pm 0$  to  $\pm 5$  per cent. The insensible water loss per hour was calculated from the insensible weight loss by subtracting the weight lost through gas exchange (weight of carbon dioxide expelled minus weight of oxygen consumed, this data being obtained from analyses of the gases expired during the measurements of the metabolic rate). The percentage of the total heat lost through water evaporation from the skin and lungs was calculated from the caloric equivalent of the water evaporated (grams water  $\times 0.58$  = calories) and the total metabolism. The average volume of the expired gases for the two 10 minute periods in which metabolic measurements were made were utilized to calculate the respiratory minute volumes.

The measurements of basal metabolic rate were made with a Collins-Benedict-Roth apparatus on the day of the measurements of insensible water loss or shortly preceding this date. The Aub-DuBois standards (15) were utilized in calculating the deviation of the basal metabolism from normal.

## RESULTS

The average metabolism of the control subjects on the balance was 65 calories per hour and the average insensible water loss was 33 grams per hour; in the hypothyroid group the average metabolism was 51 calories per hour and the average water loss only 19 grams per hour (Table I). The average basal metabolic rate of the hypothyroid patients was 29 per cent below the standard average normal with variations between minus 23 and minus 44 per cent. In the subjects without hypothyroidism an average of 29 per cent of the total heat produced was eliminated by evaporation through the skin and respiratory passages; in the hypothyroid patients an average of 22 per cent of the heat produced was thus eliminated. Whereas the percentage heat dissipated by evaporation was above 25 per cent in nine of eleven experiments in the control subjects, it was below 25 per cent in seven of the nine experiments in the patients with hypothyroidism. The results in the two hypothyroid patients whose metabolic rates were raised to plus 7 and minus 12 per cent with dinitrophenol were similar to the results obtained in the same patients before the drug was given (Table I).

<sup>2</sup> Obtained from Henry Troemner, Philadelphia, Pa.

TABLE I  
Comparison of insensible water loss and metabolism in hypothyroid patients and in normal individuals, together with related data

Case	Diagnosis	Age years	Sex	Body surface area square meters	Basal meta- bolic rate, Deviation from normal per cent	Metabolic data obtained with subject seated on balance				Remarks
						Metab- olism calories per hour	Insensible water loss grams per hour	Respiratory minute volume		Heat lost by evapo- ration per cent of total
								liters	liters per square meter body surface	
M.K.	Pernicious anemia	47	F	1.42	—	53	18	7.6	5.3	20
I.H.	Normal	61	M	1.72	—	62	27	6.6	3.9	25
R.S.	Angina pectoris	41	M	1.73	-5	73	33	7.9	4.3	26
R.St.	Angina pectoris	56	M	1.76	-15	64	29	7.4	4.3	26
F.M.	Normal	40	M	1.31	—	75	34	6.9	5.2	26
M.V. 2	Normal	25	F	1.76	-9	65	35	5.1	2.9	31
W.M. 2	Normal	22	M	1.69	-15	70	37	7.3	4.3	31
M.V. 1	Normal	25	F	1.76	-9	61	35	5.0	2.8	33
W.M. 1	Normal	22	M	1.69	-15	66	38	6.6	3.9	33
M.S.	Gout	71	M	1.72	—	68	39	8.4	4.9	33
D.G.	Normal	32	F	1.71	-21	63	37	5.5	3.2	34
Average				1.66		65	33	6.7	4.1	29
M.C.	Hypothyroidism	57	M	1.71	-23	61	16	5.1	3.0	15
G.F. 1	Hypothyroidism	53	M	1.79	-28	52	15	4.5	2.5	17
M.S.	Hypothyroidism	46	M	1.69	-25	50	18	4.2	2.5	21
S.F.	Hypothyroidism	53	M	1.91	-44	50	18	4.9	2.6	21
G.F. 2	Hypothyroidism	53	M	1.79	-28	49	18	4.6	2.6	22
R.S. 2	Hypothyroidism	57	F	1.59	-*	46	17	3.4	2.1	22
W.B.	Hypothyroidism	55	M	1.66	-27	49	20	5.8	3.5	24
W.D.	Hypothyroidism	24	M	1.73	-28	57	25	5.9	3.4	25
E.P.	Hypothyroidism	58	F	1.70	-31	46	27	4.3	2.5	34
Average				1.73	-29	51	19	4.8	2.8	22
M.S. 2	Hypothyroidism	46	M	1.68	-7	70	25	5.1	3.0	20
E.P. 2	Hypothyroidism	58	F	1.70	-12	58	29	4.7	2.8	29
										Thyroid $\frac{3}{8}$ grain daily
										Thyroid $\frac{1}{4}$ grain daily up to 2 days before test
										Thyroid $\frac{1}{8}$ grain daily
										Thyroid $\frac{1}{2}$ grain daily
										Dinitrophenol
										Dinitrophenol

\* Basal metabolic rate measurements not reliable, clinical evidences of myxedema corroborated by a cholesterol concentration of 467 mgm. per 100 cc. of serum.

The respiratory minute volume per square meter of body surface was 32 per cent lower in the hypothyroid group than in the control subjects (Table I). In the two groups of subjects the average and the range of variation of skin temperatures were practically the same at all the body sites except in the lower extremities, where the values tended to be slightly lower in the hypothyroid group. In the patients with hypothyroidism the metabolism sitting on the balance averaged 10 per cent higher than the basal metabolism; in five control subjects (7 experiments), where the basal metabolic rates were known, the metabolism sitting on the balance averaged 15 per cent higher than the basal.

#### DISCUSSION

The foregoing experiments demonstrate that the percentage of the total heat production which is eliminated by water evaporation from the skin and respiratory passages is distinctly lower in patients with hypothyroidism than in normal subjects. These experiments were performed with the subjects sitting upright on a hard surface, and the results are therefore not strictly comparable with those obtained in subjects in the basal state by other investigators; this difference in experimental conditions probably accounts for the somewhat higher average percentage heat loss by evaporation in our control group than has been found in normal subjects under strictly basal conditions by others (1, 2, 6). Benedict and Wardlaw (8) in four experiments found the insensible water loss to increase on an average of 20 per cent in the sitting position as compared with the lying position; no simultaneous metabolic measurements were made, but the increase in water loss seemed somewhat higher than the expected increase in metabolism (8).

A few experiments performed under basal conditions in patients with hypothyroidism are reported by other investigators, and our conclusions accord with their results (1, 4, 5, 9). Soderstrom and DuBois (1) found the percentage heat lost by evaporation under basal conditions in four experiments in three dwarfs with symptoms of hypothyroidism (cretins) somewhat less than normal; the values for these four experiments were 20, 21, 21 and 24 per cent heat lost by evaporation,

whereas under the same experimental conditions these authors found that normal subjects of the same age lost from 21 to 28 per cent (average 24 per cent) of their heat by evaporation. Magendanz (4) reports one strikingly low percentage heat loss by evaporation in an obese patient with symptoms of myxedema and a basal metabolic rate 30 per cent below the standard average normal; after thyroid treatment the heat lost by this channel was increased. Jores (9) found a markedly diminished percentage heat loss by evaporation from the skin in one myxedematous patient whose basal metabolic rate was 24 per cent below the standard normal; after treatment the water loss by the skin increased to normal also in this instance.

Benedict and Benedict (16) and Jores (9) have shown that in normal individuals under basal conditions approximately 40 per cent of the total water evaporated from the body comes from the respiratory passages. In our experiments the respiratory minute volume of the patients with hypothyroidism averaged 32 per cent lower than that of the control group, and the calories per hour averaged 22 per cent lower (Table I). From these results it is apparent that the volume respiration per unit of metabolism was not significantly different in the hypothyroid subjects than in the control group.<sup>3</sup> It may be concluded that the decrease in water loss in the respiratory passages in the hypothyroid group was therefore in approximate proportion to the lowering of the metabolism. Since, however, the total percentage heat lost through evaporation was low in the hypothyroid group, it follows that the percentage heat lost through loss of water by the skin must have been markedly below normal. This finding accords with Jores' (9) direct observation of a markedly decreased percentage heat loss by vaporization of water from the skin in one case of myxedema. The lowered loss of water by the skin in our patients with hypothyroidism does not appear to be due to lowered skin temperature; although the average skin temperature of the lower extremities was slightly less than that of the control group, there was no correlation between the skin tempera-

<sup>3</sup> The average respiratory volume per calorie was .103 liter in the control group and 0.094 in the hypothyroid group.

ture and the insensible water loss in individual instances. The abnormal diminution in loss of water by the skin in patients with hypothyroidism may be due to the dryness of the skin in such cases. On the other hand, some other rearrangement may be the essential factor in the lowered loss of water by the skin and the consequent conservation of body heat. In contrast to the abnormally low loss of water by the skin in hypothyroidism, Jores (11) has reported an abnormally high loss from the skin in hyperthyroidism.

The value of an indirect method of measuring total 24 hour metabolism of normal and pathological subjects under conditions of varied activity, i.e. sleep, moderate exercise, eating, talking, reading, etc., is apparent. Newburgh and his co-workers (17, 18), Laviertes (7), and others have attempted to apply measurements of insensible weight loss to this purpose. The hypotheses on which the measurement of total 24 hour metabolism by insensible water loss rest, are: (1) the percentage of the total heat which is lost by evaporation in a given normal or pathological individual in the basal state does not vary appreciably from the average figure for normal individuals; (2) the percentage heat lost by evaporation under conditions of sleep, digestion, moderate activity, etc., does not vary significantly from that under basal conditions; and (3) sensible perspiration does at no time appear. It is apparent from our results that it would be inaccurate to use the same factor for heat lost by evaporation in patients with hypothyroidism and in normal subjects. It appears, moreover, that the factor in both normal and diseased subjects varies to such an extent even under basal conditions (3, 4, 9, and 11) and with the patient sitting quietly (Table I) that the acceptance of an average factor for any group, normal or pathological, may lead to quite erroneous conclusions. As stated above, there is evidence to indicate that the percentage heat lost by evaporation in sleep, after eating, on activity, etc., is not the same as under basal conditions. There is also evidence that the percentage of the total heat production eliminated by water evaporation may be quite abnormal in obesity (4, 19, and 20), diseases associated with abnormal water balance (literature reviewed by

Laviertes (7)), hyperthyroidism (1, 4, and 11) and other conditions. Laviertes (7) has reviewed the results of other investigators who have reported measurements of 24-hour metabolism by insensible weight losses and has added observations of his own. His studies illustrate fallacies in the method. From the results of our studies it must be concluded that an empirical relationship between water loss and metabolism based on results found under basal conditions in normal individuals can not be applied to the measurement of metabolism in patients with hypothyroidism. It also seems to the authors that even though the percentage of the total heat production for a given individual were found constant under basal conditions, this value could not be applied with reliability to a measurement of total 24-hour metabolism.

TABLE II

*Skin temperature findings*

Body site	Average skin temperature		Limits of skin temperature	
	Control* group	Hypothyroid group	Control group	Hypothyroid group
	* C.	* C.	* C.	* C.
Forehead.....	32.4	32.2	31.6-33.5	31.4-32.9
Substernal notch....	32.5	32.5	31.4-34.0	30.7-34.8
Midsternum.....	32.9	32.4	31.9-34.3	31.7-33.2
Navel.....	33.1	32.5	32.5-35.0	31.1-34.2
Lower arm†.....	32.6	32.0	31.5-34.5	30.6-33.3
Palm.....	32.0	31.6	30.7-34.3	28.1-32.9
Thumb.....	30.4	30.3	27.5-32.6	26.8-32.5
Shin.....	31.4	31.0	30.2-32.7	30.5-31.6
Dorsum.....	30.7	29.4	28.6-33.5	27.3-31.1
Great toe.....	28.3	27.4	26.1-32.1	24.6-30.3

\* There were 9 experiments in 8 control subjects, and 7 experiments in 6 subjects with hypothyroidism.

† The temperatures recorded for the extremities represent the average of the mean findings for the two limbs.

## SUMMARY

The insensible water loss and the metabolism in the sitting position have been measured in patients with hypothyroidism following total thyroidectomy and, under identical experimental conditions, in normal individuals. The insensible water loss was found strikingly decreased in hypothyroidism, and the percentage of the total heat lost by water evaporation in the hypothyroid patients was usually much less than in the normal subjects. Cal-



culations indicate that the water lost through the lungs in the patients with hypothyroidism was diminished approximately in proportion to the diminished metabolism; the water lost through the skin on the other hand was diminished markedly out of proportion to the lowered metabolism. The pitfalls encountered in attempts to estimate total metabolism by measuring insensible perspiration are discussed; it is concluded that the results of metabolism studies, so made, are subject to unpredictable errors.

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# THE RESPONSE OF THE GUINEA PIG'S RETICULOCYTES TO SUBSTANCES EFFECTIVE IN PERNICIOUS ANEMIA.

## A BIOLOGIC ASSAY OF THE THERAPEUTIC POTENCY OF LIVER EXTRACTS.<sup>1,2</sup>

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If a suitable quantity of mammalian liver, or an extract thereof, be given to unselected guinea pigs, a significant increase in the number of circulating reticulocytes will ensue in the majority of the animals. This phenomenon was first observed seven years ago, and since that time considerable data have been obtained which indicate that the reticulocytosis may be utilized as a valid indicator of the therapeutic potency of materials

because of the presence of evidence of hemolysis, have been said to simulate human pernicious anemia. But to most investigators has been lost the fact that the therapeutic efficacy of liver, in human disease, is confined to pernicious anemia and related macrocytic anemias, and the fact that none of the toxic materials have induced in animals a condition which even remotely resembles Addison's anemia.

The extensive literature dealing with biological tests is summarized in Table I. Space does not permit a

TABLE I

*Summary of literature concerning biological tests for therapeutic potency of liver extracts*

	Positive results	Negative results
Normal animals.....	(11) (35) (7) (20)	(1) (34) (38) (13) (37) (14)
Animals rendered anemic by hemorrhage.....	(18)	(1) (9) (27) (36) (38)
Animals rendered anemic by infection.....	(2) (6)	(26) (29) (36)
Animals rendered anemic by dietary deficiency.....	(23) (29) (39)	(36)
Animals rendered anemic by toxic materials.....	(17) (30) (33) (27)	(1) (5) (8) (12) (13) (27)
Miscellaneous anemias.....	(19) (22)	(32) (36) (38) (17)

which are effective in pernicious anemia. A preliminary note concerning this work has already been published (40).

Many attempts have been made in the past to devise a method of biologic assay of liver extracts. As far as the author is aware, only one of the many heretofore proposed has been demonstrated fully valid.

### HISTORICAL

Most of the studies previously reported have taken the form of investigations of the effects of liver extracts upon the blood of either normal animals, or of animals rendered anemic in various ways. Several toxins have been known to produce in laboratory animals anemias, which,

critical discussion of the many proposed tests, but, in the author's opinion, none give promise of serving as a valid indicator of the therapeutic activity of substances which are effective in pernicious anemia. To this generalization there are three exceptions, which are discussed below.

Of interest is the recent communication of Landsberg and Thompson (20). These authors, independently of the present writer, found that normal guinea pigs, maintained on a normal diet, reacted to the subcutaneous administration of commercial liver extract with a reticulocytosis. Six guinea pigs, after the injection of liver extract, exhibited reticulocytoses ranging from 4 to 8 per cent. One month later, two of these animals again reacted with a reticulocytosis to the administration of liver extract. Two animals did not react to the injection of an iron salt. The authors conclude that the phenomenon is worthy of further study.

Recently Miller and Rhoads (24) have proposed a different type of test. These authors noted that five adult guinea pigs, when fed a Goldberger diet which was productive of canine black tongue, lost weight, and died in two to three weeks. Five other guinea pigs, when fed the same diet together with commercial liver extract, gained weight, and survived. A group of four animals which received the same diet together with 1 gram of

<sup>1</sup> This study was made possible by assistance from the William W. Wellington Research Fund and the DeLamar Mobile Research Fund of Harvard Medical School and by Therapeutic Research Grants Numbers 206 and 244 of the Council on Pharmacy and Chemistry of the American Medical Association.

<sup>2</sup> Presented in abstract before the American Society for Clinical Investigation, April 30, 1934.

vegex (autolyzed yeast) lost weight and died. Similar results were obtained with a group of three guinea pigs fed the diet plus 2 grams of ventriculin. But a group of four guinea pigs given the diet together with 1 gram of ventriculin and 1 gram of vegex gained weight and survived. The conclusion was reached that the death or survival of the guinea pig may serve as a useful test for evaluating the potency of various substances used in pernicious anemia.

It is the opinion of the present writer that the validity of the above phenomenon, as a test for all types of substances effective in pernicious anemia, is not yet demonstrated. In the author's laboratory the administration to guinea pigs, fed the basic diet of Miller and Rhoads, of large amounts of a more highly purified but therapeutically active commercial liver extract did not prevent the weight loss exhibited by the control animals on the basic diet alone.

The most recent attempt to produce an anemia by means of a dietary deficiency has yielded very significant results in the hands of Miller and Rhoads (39). By feeding a modified canine-black-tongue-producing diet to swine, a symptom-complex marked by oral mucous membrane lesions, achlorhydria, and macrocytic anemia, was produced. This anemia was relieved by the administration of an extract of the livers of normal swine, but not by the extract of the livers of the anemic swine. Further similarity of this pathological condition with pernicious anemia of man was evidenced by the facts that in the anemic swine, the femoral bone marrow became hyperplastic, and the gastric juice and the liver were devoid of hematopoietic activity in pernicious anemia. These findings of Miller and Rhoads suggest the use of such anemic swine as test animals for the assay of liver extracts. The quantitative relation between the amount of liver administered to the swine and the resultant hematopoietic effects remains to be investigated.

#### METHODS

##### *The care of the guinea pigs*

The animals used in the present study were adult male guinea pigs, weighing, at the time of acquisition, between 300 and 800 grams. They were obtained from various sources. No reason, other than the desirability of avoidance of pregnancy, accounts for the use of only male animals. The animals are maintained in small cages, each holding from six to eight guinea pigs, and each fitted with a wire bottom, the holes of which are large enough to eliminate coprophagy. The diet of the animals consists solely of oats, carrots, and lettuce. The quantity of food is not measured. No hay, sawdust, or wood shavings are present in the cages.

##### *The method of estimation of reticulocytes*

For the purposes of this study the following method has been found most satisfactory, affording a high degree of accuracy with the expenditure of a minimum amount of labor. A clean glass slide is warmed over a flame and is then coated with a thin film of saturated alcoholic solution of brilliant cresyl blue. The ear of the guinea pig is wiped off with alcohol, and a very small drop of capillary blood is taken up on a clean cover slip, which is then inverted upon the stained slide. The blood film is spread out by gentle pressure on the cover slip, and then the sides of the cover slip are rimmed with melted paraffin. This preparation is suitable for the estimation of reticulocytes for not longer than forty-five minutes, for after the lapse of a longer interval excessive hemolysis takes place. The eyepiece of the microscope is blocked off, with holes cut in paper, so that each oil immersion field contains from twenty-five to thirty erythrocytes. The reticulocytes in this wet preparation are readily identified; all red blood cells containing either punctate or thread-like particles of purple staining reticulum are classified as reticulocytes. The reticulum can readily be distinguished from platelets or from particles of the dye by the position of the latter outside the erythrocyte. The counting is carried out in areas in which the erythrocytes are distributed in a fairly uniform and unicellular layer. The reticulocytes among two hundred and fifty red blood cells in one corner of the preparation are counted, and a like number in an opposite corner; the number of reticulocytes observed multiplied by 0.2 yields the per cent of reticulocytes.

The above method of estimation of reticulocytes has been described in detail, for it is the opinion of the author that repetition of the studies reported in this paper requires the exact duplication of the author's technique.

Comparative reticulocyte counts by the wet method and by the conventional method, on a dry smear counterstained with Wright's stain, performed on samples of blood from nine guinea pigs, yielded no significant differences in the percentage of reticulocytes. The accuracy of the wet method, in the hands of trained observers, was demonstrated by the following experiment. Two observers, independently of one another, counted

the reticulocytes in six different areas, each area comprising 500 erythrocytes, of samples of blood from five guinea pigs. A highly uniform distribution of reticulocytes in different parts of the wet preparation was observed. The greatest differences in distribution were of the same order of magnitude as the greatest differences between the counts of the two observers. In neither case was the divergence large enough to render invalid the differentiation between a negative and a positive reticulocyte response (*vide infra*).

Further evidence for the reliability of the reticulocyte method is furnished by the relative constancy, in the individual animal, of the negative responses presented in Tables III and V.

#### *The method of administration of liver extracts*

Unless otherwise stated, the experiments to be described below concern the effects of substances introduced parenterally. All solutions, containing water as the diluent, are made up to a volume of approximately 5 cc., and are injected intraperitoneally, without any aseptic or antiseptic precautions. No peritoneal inflammatory reaction following such an injection has ever been noted.

Substances which are to be administered orally are best given in a volume not exceeding 2 cc.

#### *The selection of reactive guinea pigs*

*The reticulocyte levels of unselected guinea pigs.* The reticulocyte counts of thirty unselected guinea pigs were studied over a period of eight successive days after the installation of the animals in the environmental conditions described above. It was observed that the initial reticulocyte counts of many animals were above 1.2 per cent, that during the first four days many counts remained above this level, but that, with the exception of three animals, during the last four days the reticulocyte counts remained at 1.2 per cent or lower. It has been the practice of the writer to discard guinea pigs which exhibit fluctuant reticulocyte levels during the first week of observation.

The animals which exhibit stable reticulocyte counts of not over 1.2 per cent, which comprise the majority of all newly acquired guinea pigs, are then tested for their capacity to react with a reticulocytosis to the administration of therapeutic

tically active liver extract. For this purpose commercial liver extract,<sup>3</sup> in an amount derived from 4.3 mgm. of fresh liver per kilogram weight of guinea pig, is injected intraperitoneally, on one occasion only. During the subsequent six days a large proportion of the injected animals will exhibit a positive reticulocyte response, in a manner to be described fully below. The proportion of animals which are found to be reactive, on the initial test, varies amongst different batches of guinea pigs; from 30 to 70 per cent is the proportion most often encountered. Only those guinea pigs which react to the initial test with a positive response are retained for further experimental purposes.

The uninjected guinea pigs offer no clue that might serve to differentiate reactive from non-reactive animals. Neither the weights of the animals, the height of the erythrocyte counts, the initial reticulocyte levels, or the appearance of the stained blood smear, show any significant differences between the two classes.

The variations in the proportions of reactive animals do not seem to bear any relation to the season of the year.

It has been the practice of the author, in the past, to discard those animals which have not reacted to the initial test with a reticulocytosis. It is not to be inferred, however, that there necessarily exists a fundamental qualitative difference between the two classes of guinea pigs. For, further tests for reactivity of the initially non-reactive animals may remove a large proportion from this class. In Table II are presented the results of the retesting, at approximately monthly intervals, of a group of twenty-five unselected

TABLE II  
*Responses of 25 unselected guinea pigs to successive retesting*

Number of animals	7	6	7	3	2
Mgm. of fresh liver injected per kilo of animal					
43.0.....	+	—	—	—	—
43.0.....	+	+	—	—	+
4.3.....	+	+	+	—	+
4.3.....	+	+	+	—	+
4.3.....	+	+	+	+	—

<sup>3</sup> The author has used Solution Liver Extract-Lederle, N. N. R.

animals. It is evident that the seven guinea pigs which were initially reactive remained reactive through the subsequent tests; while of the initially non-reactive animals, thirteen became reactive and exhibited positive responses on three or more successive occasions. Of the remaining five initially non-reactive animals, three became responsive on the fifth test, while only two did not remain consistently reactive after the first positive response. In no case, therefore, did any animal constantly exhibit a non-reactive state.

*The hematopoietic response to the administration of liver extracts*

*The reticulocyte response.* In order to study the reticulocyte level during a control period, counts on twenty-seven reactive guinea pigs (i.e., those animals which have reacted to the initial test with a positive response) were performed on six successive days, during which nothing was administered. Only one animal exhibited values as high as 1.6 per cent reticulocytes. A statistical treatment of these data yields the following results:

Arithmetical mean ..... 0.85 per cent reticulocytes  
Standard deviation ..... 0.33 per cent reticulocytes  
Probable error ..... 0.22 per cent reticulocytes  
Probable error  $\times 3.2$  ..... 0.70 per cent reticulocytes

It is fair to conclude, therefore, that practically all resting, reactive guinea pigs, over a period of six successive days, will not exhibit reticulocyte counts greater than 1.55 per cent.

On the other hand, following the administration of therapeutically active liver extract, the reticulocytes almost invariably, during the subsequent six days, rise to at least 2.0 per cent. Numerous examples of these responses are to be seen in Table III. A *positive response* is defined by the author as a rise of the reticulocyte count to at least 2.0 per cent on two successive days, within six days after the administration of the substance to be tested. A *weakly positive* or *doubtful* response is defined as a rise of the reticulocyte count to at least 2.0 per cent on only one day, or on two non-successive days, of the six day period. All other reactions, within the six day period, are termed *negative* responses.

Many examples of weakly positive and of negative responses are contained in the tables cited above.

TABLE III  
*Responses of erythrocytes and of absolute number of reticulocytes*

Guinea pig number	Negative responses				Guinea pig number	Positive responses			
	Days after injection	Reticu- lo- cytes	Red blood cells	Num- ber of reticu- lo- cytes		Days after injection	Reticu- lo- cytes	Red blood cells	Num- ber of reticu- lo- cytes
		per cent	mil- lions				per cent	mil- lions	
297	1	1.0	6.10	61,000	42	1	1.2	4.99	60,000
	2	0.2	6.27	12,500		2	1.8	6.24	112,400
	3	0.8	6.21	49,700		3	1.8	6.64	119,600
	4	1.2	6.05	72,600		4	3.4		
	5	0.8	6.39	51,200		5	2.2	4.95	109,000
	6	0.6	6.42	38,500		6	2.6	4.94	128,500
31	1	0.4	5.21	20,800	296	1	1.6	6.30	101,000
	2	0.8				2	0.8	4.97	39,700
	3	1.2	5.48	65,800		3	1.2	5.03	60,800
	4	1.0	5.76	57,600		4	0.2	4.94	9,900
	5	0.8	5.91	47,250		5	2.0	4.60	92,000
	6	0.8	5.79	46,300		6	1.8	4.75	85,050
48	1	0.8	5.73	45,800	298	1	2.4	4.84	116,000
	2	0.6				2	2.6	5.38	140,000
	3	0.6	6.54	39,200		3	1.0	5.15	51,500
	4	0.6	6.52	39,100		4	1.4	5.70	80,000
	5	0.4	6.58	26,300		5	2.0	6.54	113,000
	6	0.2	6.31	12,600		6	2.8	5.56	156,000
40	1	0.6			39	1	2.4	6.18	148,000
	2	1.4	5.80	34,800		2	1.0	5.72	57,200
	3	1.4	5.87	82,200		3	1.6	5.43	86,800
	4	1.6	4.96	78,400		4	1.4		
	5	1.6	5.70	91,200		5	1.0	4.75	47,500
	6	1.6	5.66	90,600		6	2.0	5.35	107,000
114X	1	1.8	5.40	97,200	63	1	2.0	6.24	124,800
	2	1.0	6.83	68,300		2	2.2	5.70	125,000
	3	1.0	6.19	61,900		3	0.8	6.58	52,600
	4	1.4	6.56	91,800		4	0.6		
	5	1.4	6.77	94,800		5	2.6	6.72	175,000
	6	1.4	6.50	91,000		6	2.2	6.40	141,000
131X	1	0.8	6.47	51,800	131X	1	2.4	7.21	173,000
	2	0.8	6.59	52,700		2	2.4	6.80	163,000
	3	0.8	5.83	46,700		3	2.0	6.60	132,000
	4	0.4				4	1.8	7.05	127,000
	5	0.4				5	0.4	6.08	24,300
	6	0.4				6	0.2	5.21	10,400
114X	1	0.4			131X	7	1.0	5.60	56,000
	2	0.4				8	1.0	5.44	131,000
	3	0.4				1	2.0		
	4	0.4				2	2.4		
	5	0.4				3	2.0		
	6	0.4				4	2.4		
114X	1	0.4			131X	5	2.4	6.56	157,000
	2	0.4				6	1.0	7.74	77,400
	3	0.4				7	1.4	7.68	107,000
	4	0.4				8	1.4		
	5	0.4				1	0.4		
	6	0.4				2	0.2		

That the reticulocytosis is both relative and absolute is demonstrated by the protocols of Table III.

The peak of the reticulocytosis is always attained within six days after the administration of the liver extract, but the reticulocytosis of at least 2.0 per cent persists for several days, and the values recede, in an irregular fashion, to the control level of 1.2 per cent, or less, within ten to twenty-one days, in most instances.

It is an invariable practice in this laboratory to await the return of the reticulocyte count to 1.2 per cent or less, before another experiment is undertaken. Values of 1.6 or 1.4 per cent may be followed by a slight transient rise. On the

other hand, the return of the reticulocyte count to at least 1.2 per cent insures a low level during a subsequent six day period.

The above definition of a positive response is an arbitrary one, and, to be sure, necessarily implies the distinction between a reticulocyte count of 2.0 per cent and one of 1.8 per cent. It is not the contention of the writer that such a distinction is possible. Fortunately, the necessity of making such a fine distinction is rarely encountered. In Table IV are presented the frequency distribution

of the experiment on another animal; only *positive* or *negative* responses, as defined above, are to be accepted. Doubtful responses are not repeatedly observed in any one animal.

To the infrequency of reticulocyte peaks which fall on the border line between a positive and a negative response, and to the necessity of repetition of every doubtful reaction, is to be added another safeguard of the validity of the observation, namely, the procedure used in the quantitative assay, whereby a series of graded amounts

TABLE IV  
*Frequency distribution of reticulocyte peaks*

Per cent reticulocytes	0.6	0.8	1.0	1.2	1.4	1.6	1.8	2.0	2.2	2.4	2.6	2.8	3.0	3.2	3.4	3.6	3.8	4.0	4.2	5.4	5.8	6.2	7.6
Negative responses (131 experiments).....	4.4	16.6	17.4	24.2	19.1	12.2	6.1																
Positive responses (193 experiments).....								2.7	8.9	10.4	6.8	10.3	17.6	19.7	11.4	5.7	1.6	1.6	0.5	0.5	0.5	0.5	1.0
Doubtful responses (30 experiments).....								37.0	23.1	10.0	0.0	6.5	6.7	6.7	6.7								

of the reticulocyte peaks of a large number of experiments. These data are derived from 354 experiments on 115 reactive guinea pigs, following the administration of a large variety of materials. The reticulocyte responses are classified as follows: Positive, 55 per cent, negative, 37 per cent, and weakly positive or doubtful, 8 per cent. It is evident that among the negative responses, the reticulocyte peak was *under* 1.8 per cent in 93.9 per cent of all cases, and in 81.7 per cent of all instances was *under* 1.6 per cent. Similarly, of the positive responses, 88.4 per cent of all peaks were *over* 2.2 per cent, and 68.0 per cent were *over* 2.4 per cent. Thus, in approximately 82 per cent of all responses, following the administration of a variety of materials, the borderline values of 1.8 to 2.2 per cent reticulocytes may not be expected to present themselves. The distribution of the peaks of the weakly positive or doubtful responses are illustrative of this interpretation. Thus, inasmuch as the summit of the distribution curve is found at a reticulocyte count of 2.0 per cent, with a steep downward slope, it seems fair to conclude that these values of 2.0 and 2.2 per cent reticulocytes, observed on only one day of the six day period, are an expression of the unavoidable error inherent in the method of estimation of the reticulocytes. For this reason, every doubtful result necessitates the repeti-

tion of the experiment on a moderately large number of animals. This procedure will be described below.

The reticulocyte response of the reactive guinea pig appears to be an all-or-none reaction. There is no relation between the amount of liver administered, above a minimal effective dose, and the height of the subsequent reticulocyte peak (Fig. 1). Regardless of the dosage, 97 per cent of all reticulocyte peaks of positive responses are distributed between 2.0 per cent and 4.0 per cent reticulocytes, inclusive (Table IV). On the other hand, the data of Figure 2 suggest a slight tendency of the reticulocyte peak to appear earlier in the six day period, with increasing amounts of liver material administered.

There is no correlation between the height of the reticulocyte peak and the initial erythrocyte count of the animal.

The reticulocytosis that the *oral* administration of liver extract induces differs qualitatively in no way from that following the intraperitoneal administration.

As far as the present data indicate, the reactive state is maintained indefinitely. The animal which has been in this laboratory the longest period has exhibited a total of 18 positive responses during the past 21 months. Another animal, during the 16 months preceding death, reacted positively 14

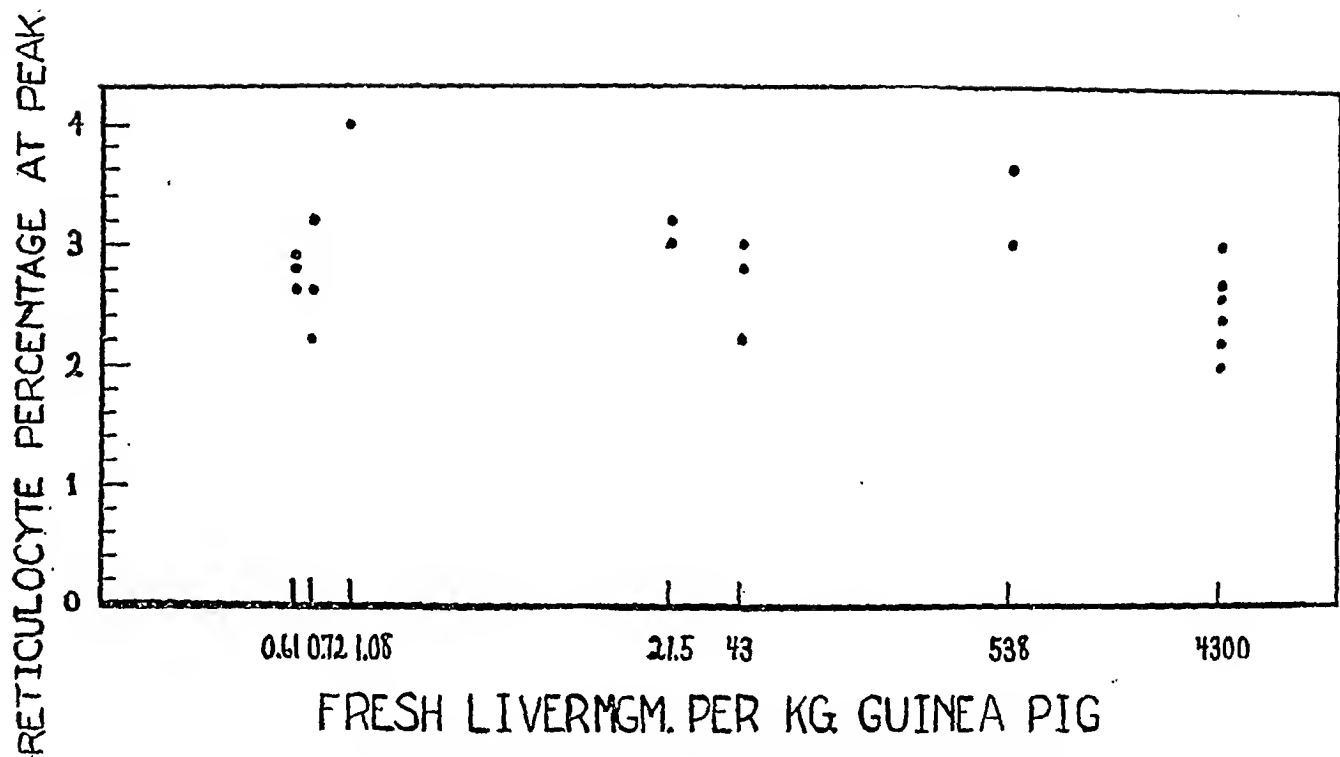


FIG. 1. THE RELATION BETWEEN THE DOSAGE OF LIVER EXTRACT AND THE MAGNITUDE OF THE RETICULOCYTE RESPONSE

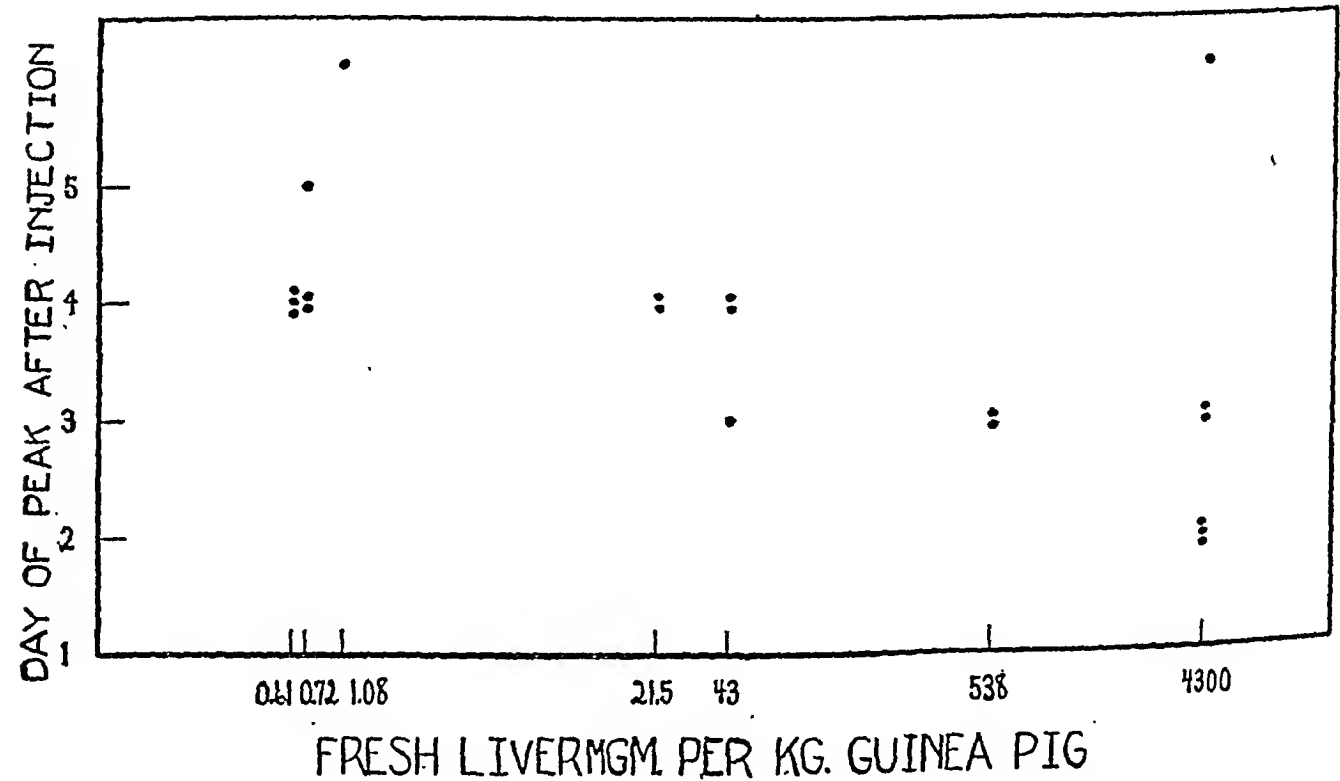


FIG. 2. THE RELATION BETWEEN THE DOSAGE OF LIVER EXTRACT AND THE TIME OF THE RETICULOCYTE RESPONSE

times. On retesting 15 reactive guinea pigs, all of which had exhibited reactivity on at least 4 occasions over a period of 4 months or longer, 14 positive responses were observed. One guinea pig, which reacted negatively, showed 6 subsequent positive responses during the following 5 months.

*The erythrocyte response.* The erythrocyte responses to the administration of several inert ma-

terials, to five reactive guinea pigs, and of several active materials, in various amounts to nine animals, are depicted in Table III. The averages of all counts in each group are as follows:

	Erythrocytes	
	Negative response million per cu. mm.	Positive response million per cu. mm.
Day of injection .....	6.24	5.64
Day after injection		
1 .....	6.07	5.52
2 .....	6.26	5.77
3 .....	6.05	5.53
4 .....	6.14	5.56
5 .....	6.07	5.87
6 .....	5.73	6.10
7 .....		6.81

The only inference from these scanty data that can be drawn is that the erythrocytes of the positively reacting animals tend to rise slightly, after the fourth day following the administration of liver material. A more complete investigation of these changes is under way.

#### *The quantitative assay of the hematopoietic activity of liver extracts*

##### *Minimal effective dose or standardization curve.*

The quantitative application of the induced reticulocytosis involves the definition of the unit of hematopoietic activity. This unit might be arrived at by one of two different procedures. If the assumption is made that all unselected guinea pigs are of an approximately equal degree of sensitivity, and that each individual animal may exhibit, in successive tests marked variation in degree of sensitivity, it should be necessary to follow the procedure described by Gaddum (10) and applied to the assay of oestrin by several workers (4, 21). This method consists of the construction of a curve which depicts the percentage of positive reactions among a large number of animals as a function of several different doses of oestrin; the amount of oestrin which induces oestrus in 50 per cent of the animals is termed the unit. In a similar fashion, a material of unknown potency is administered to a large number of animals, and by reference to the standardization curve the number of units in the material is ascertained.

Table II contains data bearing on this question. Of the twenty-five unselected, newly acquired guinea pigs initially injected with material derived from 43 mgm. of liver, 36 per cent exhibited a

positive response; while after the second administration of the same amount, this proportion rose to 68 per cent; and a third test, with *one-tenth* of the previous dosage, yielded 92 per cent of positives. But if the concept of a standardization curve be applicable, one would expect that the percentage of positive responses in the second test would be approximately equal to that in the first test; and that the proportion of positive responses in the third test would drop considerably. On the other hand, a similar increase in the percentage of positive responses during the second testing occurred in one group of unselected animals injected on each occasion with liver extract from 4.3 mgm. of liver. These observations demonstrate that all unselected guinea pigs, at the time of the initial test, are not equally sensitive; while a large amount of data have demonstrated that guinea pigs which have reacted positively to the initial test maintain, indefinitely, an approximately equal degree of sensitivity.

The hypothesis that a majority of reactive guinea pigs respond to the administration of a *minimal effective dose* of liver material in a consistent fashion has been rendered valid by the results of over one thousand experiments on several hundred animals, with a large variety of liver extracts. This method of assay will be described below.

*The definition of the unit of hematopoietic activity.* In Table V are presented the protocols of the assay on guinea pigs of a therapeutically potent, commercial liver extract. The potency of this lot of extract was ascertained on two patients suffering from pernicious anemia. In each case the resultant hematopoietic response was indicative of a high degree of therapeutic effectivity (Table VIII, second periods of Patients 5 and 6). In Table V it is seen that decreasing doses of this extract were administered, on one occasion only, to groups of two or three reactive guinea pigs. All the animals injected with amounts exceeding 0.54 mgm. (of fresh liver, per kilogram of guinea pig) reacted positively; one of three animals was positive to 0.54 mgm.; while smaller doses were inert. These data illustrate the method used in this laboratory for the assay of a liver extract. All weakly positive or doubtful responses, as described above, call for the repetition of the same dose in another animal; and a majority of three definitive



tration of extrinsic factor alone is devoid of effect; and, in both, the administration of intrinsic factor that has been inactivated by heat is also without effect.

It seems to the writer that the results of the foregoing experiments furnish more evidence for the view that the reactive guinea pig responds with a reticulocytosis to the same materials that are effective in pernicious anemia.

*A comparison of the guinea pig assay, and of the therapeutic potency, of liver extracts*

In Table VIII are presented the data of the assays, on guinea pig and on patient, of seven different, experimentally produced, partially purified liver extracts. These materials were administered by intramuscular injection to previously untreated patients suffering from classical pernicious anemia. In most cases the material from not more than 100 grams of fresh liver was administered, but in an amount sufficiently large to possibly induce a maximal hematopoietic effect.

It has already been demonstrated (Table V) that one particular commercial liver extract, which was therapeutically potent, exhibited on guinea pig assay an activity of 164,000 G.P.U. per 100 grams of fresh liver. Assays of nine different lots of commercial liver extracts yielded values ranging from 140,000 to 305,000 G.P.U., per. 100 grams of fresh liver. It is not the writer's intention to discuss, at this time, the possible significance of the variations in the degree of activity which these different extracts exhibit, but only to point out that 7 of these 9 extracts assayed between 164,000 and 210,000 G.P.U.

The foregoing data bear on the interpretation of the assays of extracts number 114 and number 118 (Table VIII).<sup>4</sup> Both extracts yielded the same guinea pig value of 47,000 G.P.U., that is, the amount of activity that might be derived from 25 to 35 grams of fresh liver. The very similar results yielded by both patients, to whom this extract was administered, clearly indicate that a markedly sub-maximal effect was induced. As far as the author is aware, the only observations of the hematopoietic effects in pernicious

<sup>4</sup> The author is indebted to Dr. Randolph West for the material and the clinical data of extracts number 232, 233, 234, and 252; and to Dr. William B. Castle for the material and clinical data of extract B.

TABLE VIII  
*Comparative assays on guinea pigs and on patients of liver extracts*

Liver extract number	B										100	107	234	125	232	139	114	118	252	170	233
Guinea pig assay, in terms of G.P.U. per 100 grams fresh liver. . . . .	Less than 77										375	375	Less than 2,300	Less than 2,300	Less than 15,000	Less than 23,000	47,000	47,000	At least 140,000	164,000	164,000
Patient number. . . . .	13	14	15	2.58	1.96	2.49	1.23	1.80	1.80	1.80	375	375	11	2	10	1	3	4	12	3	10
Red blood cells at beginning of experimental period. . . . .	2.50	2.05	2.40	1.4	1.2	1.4	1.09	1.88	1.70	1.43	1.70	1.70	5	6	5	4.4	10.8	14.2	33.1	19.0	72.4
Red blood cells at termination of experimental period. . . . .	8	10	8	8	8	8	11	8	5	10	11	5	10	5	5	13	12	9	7	11	10
Reticulocyte peak, per cent. . . . .	—	—	—	—	—	—	—	—	100	100	170	200	100	100	275	60	100	100	215	100	210
Length of experimental period, days. . . . .	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Total amount of fresh liver, from which extract derived, grams. . . . .	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Response to subsequent administration of commercial liver extract	Good response in each case										Same period as for extract number 100										
Red blood cells at beginning of period	1.09	2.08	2.28	1.88	1.70	1.43	1.70	1.70	1.70	1.43	1.70	1.70	1.70	1.72	See ext. no.	2.14	1.62	1.75	1.70	2.37	—
Red blood cells at end of period. . . . .	23.2	19.0	8	2.28	2.20	22.0	2.20	2.20	2.20	22.0	2.20	2.20	2.20	10	233	13.4	2.46	2.59	1.90	3.30	—
Reticulocyte peak. . . . .	11	7	7	7	7	7	7	7	7	10	7	7	7	10	10	10	10.6	10.6	7.3	8.0	—
Amount of fresh liver, from which extract derived, grams. . . . .	70	50	50	50	50	50	50	50	50	100	100	100	100	100	233	10	10	15	6	9	—

anemia of the parenteral administration of small amounts of liver are those of Strauss and Castle (31). The erythrocyte and reticulocyte responses which these authors observed subsequent to the administration of liver extract derived from 20 to 30 grams of fresh liver exhibit variations in a range into which fall the responses induced by extracts number 114 and number 118.

It is the author's conviction, based on an adequate experience in the attempt to assay liver extracts on patients, that a closer correlation of the guinea pig and of the human potency of extracts number 114 and number 118 cannot be expected.

The assays of the remaining extracts yield corresponding activities on the guinea pig and on the patient.

#### DISCUSSION

The foregoing data describe the phenomenon of the induced reticulocytosis exhibited by the reactive guinea pig, and demonstrate the application of this phenomenon to the quantitative determination of the therapeutic activity of liver preparations. The factors which condition the procedure include (1) a particular and fixed diet and environment of the guinea pigs; (2) the attainment by the observer of a high degree of accuracy in the estimation of the reticulocytes; (3) the rejection of guinea pigs the reticulocyte counts of which fluctuate, during a preliminary control period; and (4) the use, in tests of guinea pigs for reactivity, of liver extract of a high degree of therapeutic potency.

As far as the author is aware, the guinea pigs which are found to be reactive do not suffer from any disease acquired prior to the initial test, or from a pathological state induced by the dietary and environmental conditions under which the animals are maintained in this laboratory. The erythrocytes of the reactive guinea pigs do not show any resemblance to those observed in pernicious anemia, nor are the animals anemic. On the other hand, there are two facts which bear on this question. In the first place, both reactive and initially non-reactive guinea pigs possess a femoral bone marrow which is exceedingly rich in primitive elements of both the white and red blood cell series. The large number of megaloblasts, in proportion to the number of normoblasts, simulate

the classical picture of the bone marrow findings in pernicious anemia.

In the second place, it has been demonstrated that the reactive guinea pig forms reticulocytogenic material from a source of extrinsic factor only in the presence of active intrinsic factor, a phenomenon which, as far as the writer is aware, has been observed only in pernicious anemia.

The facts known at present concerning the guinea pig phenomenon do not permit any further speculation on a possible deficiency state simulating that considered to exist in pernicious anemia.

Regardless, however, of the obscurity of the basis of the phenomenon, the data presented in this study furnish evidence for the conclusion that the guinea pig test is a valid indicator of the therapeutic efficacy of materials which are effective in pernicious anemia.

#### SUMMARY

From thirty to seventy per cent of guinea pigs, maintained on a diet of oats, carrots, and lettuce, exhibit a significant rise in the number of reticulocytes following the administration to them of therapeutically active liver material. For every active material there exists a minimal effective dose, which is termed the Guinea Pig Unit of hematopoietic activity, and which is a quantitative expression of a degree of activity. Evidence of an indirect nature is presented that the capacity to induce the reticulocytosis is confined to materials which are effective in pernicious anemia, and that the guinea pig test is a valid indicator of the therapeutic potency of liver preparations.

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# THE ASSAY ON GUINEA PIGS OF THE HEMATOPOIETIC ACTIVITY OF HUMAN LIVERS, NORMAL AND PERNICIOUS ANEMIA.<sup>1, 2</sup>

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In the foregoing communication (1), it was pointed out that the indirect evidence for the validity of the assay on guinea pigs of liver extracts of their therapeutic potency in pernicious anemia must rest upon a more crucial experimental basis than upon the fact that a moderately large number of various therapeutically inert materials are likewise inert in the guinea pig. As long as the material in liver, which exerts the therapeutic effect, remains unavailable in pure form for testing in the guinea pig, a different type of experiment must be resorted to. Such an experiment is suggested by the observations of Richter, Ivy, and Kim (2); of Goldhammer, Isaacs, and Sturgis (3), and of Wilkinson and Klein (4). The results of all of these observations are identical, and may be summarized as follows: (1) the extract of the liver of a patient dying of disease other than pernicious anemia is therapeutically effective in pernicious anemia; (2) the extract of the liver of a patient treated for pernicious anemia, but dying, while in partial remission, of other disease, is almost as effective as the normal liver in the treatment of pernicious anemia; (3) the extract of the liver of a patient dying in relapse of pernicious anemia is totally inert in the treatment of this disease.

## METHODS

The livers of four patients were obtained, and were treated in an identical fashion. Each liver was finely ground, thoroughly mixed with an equal weight of 95 per cent alcohol, and dried on the steam bath about 20 hours. The resultant dry liver was reduced to a fine

powder. In order to secure an extract of the tissue which would contain practically all of the nonprotein nitrogenous substances, a crude extract was made by the Schenk method. To the powdered liver derived from 100 grams of fresh liver were added 100 cc. of normal hydrochloric acid and 100 cc. of a saturated solution of mercuric chloride. The extraction took place overnight in the refrigerator, the precipitated protein was then filtered off, and the filtrate decomposed by hydrogen sulfide. The total nitrogen content of the several Schenk filtrates varied from 400 to 450 mgm. per 100 grams of fresh liver.

## Pathologic material

*Patient Number 1.* Age 62. Diagnoses: Arteriosclerotic heart disease, cerebral arteriosclerosis, obstructing prostate, acute urinary retention. The liver was obtained 24 hours *postmortem*, and weighed 1520 grams.

*Patient Number 2.* Age 76. Diagnoses: Pernicious anemia, lymphosarcoma, bilateral hydrothorax, generalized arteriosclerosis. The liver was obtained 36 hours *postmortem*, and weighed 1470 grams.

The patient first entered the hospital thirteen months before death complaining of weakness and of generalized glandular enlargement. A biopsy of a lymph node revealed the presence of lymphosarcoma. He was discharged following satisfactory treatment with deep roentgen radiation. Seven months before death, he re-entered the hospital complaining of recurrent attacks of sore tongue, gastro-intestinal disturbances, weakness, and paresthesias of the extremities. The physical examination revealed a lemon-yellow color of the skin and sclerae, Hunterian glossitis, generalized glandular enlargement, and impaired vibration sense in the lower extremities. The blood smear was typical of pernicious anemia; red blood cells 1,360,000, oxygen capacity 8.19 volumes per cent, cell volume 19.1 per cent, volume index 1.65, color index 1.56. Achlorhydria after histamine. After the intramuscular administration of liver extract derived from 100 grams of fresh liver, the reticulocytes rose to 32 per cent on the sixth day, and the erythrocytes rose from 1,290,000 to 2,070,000 on the twelfth day. After continued liver therapy during the following three months, the erythrocytes rose to 4,670,000, and the oxygen capacity to 15.5 volumes per cent. One month before death the patient re-entered the hospital because of recurrent glandular enlargement. Red blood cells 3,270,000 at time of entry, 3,350,000 five days before death. During this period, the patient received, at intervals of three to five days, a total

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<sup>2</sup> Presented in abstract before the American Society for Clinical Investigation, April 30, 1934.

of nine intramuscular injections, each of liver extract derived from 100 grams of liver.

*Patient Number 3.* Age 76. Diagnoses: Pernicious anemia, pulmonary edema, acute cardiac failure, coronary sclerosis.

The liver was obtained 22 hours *postmortem*, and weighed 1530 grams.

The day before death the erythrocytes were 1,000,000, oxygen capacity 5.25 volumes per cent. The patient received, intramuscularly, liver extract derived from 200 grams of liver three and one-half hours before death.

*Patient Number 4.* Age 66. Diagnoses: Pernicious anemia, arteriosclerotic heart disease, congestive heart failure, auricular fibrillation, bronchopneumonia. The liver was obtained 10 hours *postmortem*, and weighed 1460 grams.

Two days before death the erythrocytes numbered 800,000, oxygen capacity 5.05 volumes per cent. During the 36 hours preceding death, the patient received by the intramuscular route, liver extract derived from 180 grams of liver; and the day before death a transfusion of 700 cc. of citrated blood.

The above four liver extracts were assayed in guinea pigs by the method described in the preceding communication (1). The protocols of these assays are presented in the accompanying table. It is evident that the livers of the patients

dying of pernicious anemia contain only a negligible amount of reticulocytogenic material and that the liver of the patient dying in partial remission of pernicious anemia contains an appreciable quantity of such material, but far less than is contained in the non-pernicious anemia liver. These results are in harmony with the assays of similar livers on patients suffering from pernicious anemia (*vide supra*).

#### SUMMARY

Indirect evidence for the validity of the guinea pig method of assay of the therapeutic potency of liver extracts in pernicious anemia is furnished by the fact that crude extracts of human livers, when assayed in guinea pigs, yielded the following number of guinea pig units, per 100 grams of fresh liver: pernicious anemia in relapse, 650 and 348, respectively; pernicious anemia in partial remission, 47,000; non-pernicious anemia, 127,000.

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TABLE I  
*Assays of human livers*

Patient number 1. Control subject								
Amount of fresh liver from which extract derived, mgm. per kilo pig.....	1.08	0.86	0.79	0.72	0.61	0.54	0.48	
Response.....	+++	+++	+-	±--	±--	---	---	Assay: 100,000 mgm./0.79 mgm. = 127,000 G. P. U. per 100 grams fresh liver
Patient number 2. Pernicious anemia in remission								
Amount of fresh liver from which extract derived, mgm. per kilo pig.....	2.15	1.72	1.44	1.08				
Response.....	+++	±±	---	---				Assay: 100,000 mgm./2.15 mgm. = 47,000 G. P. U. per 100 grams fresh liver
Patient number 3. Pernicious anemia in relapse								
Amount of fresh liver from which extract derived, mgm. per kilo pig.....	430	215	154	108	86	43	21.5	15.4
Response.....	+++	+++	+++	+-	---	---	---	---
								Assay: 100,000 mgm./154 mgm. = 650 G. P. U. per 100 grams fresh liver
Patient number 4. Pernicious anemia in relapse								
Amount of fresh liver from which extract derived, mgm. per kilo pig.....	430	287	251	215	172	43	22	
Response.....	+++	±±±	±--	---	---	---	---	Assay: 100,000 mgm./287 mgm. = 348 G. P. U. per 100 grams fresh liver

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# HETEROPHILE ANTIBODIES IN PNEUMONIA<sup>1</sup>

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Special significance has been ascribed recently to heterophile antibody in relation to pneumococcus infections. It has been suggested that the variable amounts of heterophile antibody in human sera may have some relationship to lobar pneumonia in man. The purpose of this communication is to record the results of tests for sheep cell hemolysin in the sera of patients with pneumococcus lobar pneumonia and to correlate these results with the course of the disease. The results of similar tests in normal human subjects and in subjects receiving injections of various pneumococcus antigens are included for comparison. It was hoped to shed some light on the relationship, if any, of heterophile antibody to the course of lobar pneumonia in man.

Bailey and Shorb (1, 2, 3,) showed that rabbits injected with a large number of cultures of pneumococci of different types develop a potent anti-sheep hemolysin in their sera. This antibody could be removed from these sera by absorption with homologous or heterologous boiled pneumococci or with boiled sheep red blood corpuscles. All the strains of the different types of pneumococci which they tested, with the exception of some Type III strains, possessed this property of combining with the heterophile antibodies from antisera for homologous and heterologous pneumococci and other heterophile antigens. Rabbits immunized with sheep red blood corpuscles were shown to be relatively resistant to intravenous infection with Type I pneumococci. In experiments with the dermal pneumococcus infection of Goodner in rabbits (4), Powell, Jamieson, Bailey and Hyde (5) found that the usual mouse protective antibody is very much more effective

therapeutically when fortified with heterophile antibody. This was particularly true when the heterophile antibody was incited by injections of pneumococci in rabbits. For example, they obtained equal curative effects in Type I infections in rabbits with the following: (1) 500 mouse protective units of Felton's antipneumococcus horse serum, (2) less than 5 mouse protective units and 500 heterophile units in rabbits' antipneumococcus serum, and (3) a pooled serum containing 100 mouse protective units of Type I antibody from horse serum and 500 heterophile units of rabbit antish sheep hemolysin.

It was argued by these investigators that since pneumococci have a marked affinity for heterophile antibodies, and sensitization of pneumococci with this antibody, either *in vivo* or *in vitro*, influences the course of infection of rabbits with these organisms, it would seem reasonable to suppose that the variable amounts of heterophile hemolysin in human sera influence the course of lobar pneumonia in man. The larger the amount of such antibody present, the greater should be the resistance to primary invasion and growth in the tissues of the host.

In their earlier work, Bailey and Shorb (1) reported the finding of a definite increase in sheep-cell hemolysin in the sera of cases of lobar pneumonia which recovered. They considered this to be a specific reaction to pneumococcus infection. Although these latter findings are considered highly significant and are frequently referred to, the data in only one case are mentioned as an example. In this "one case infected with an organism reported as a Type IV pneumococcus, the titer of the serum on the fifth day of the disease was 16 units per cc. and at the end of the twelfth day (sixth afebrile) the serum contained 100 units of anti-sheep hemolysin per cc." Data on the titer of sheep cell hemolysin in the sera of a considerable number of patients with lobar pneumonia are not available in the literature.

<sup>1</sup> This study was aided, in part, by a grant given in honor of Francis Weld Peabody by the Ella Sachs Plotz Foundation, and, in part, by a grant from the Influenza Commission of the Metropolitan Life Insurance Company.

## METHOD AND MATERIALS

*Titration of sheep cell hemolysin* were carried out as follows: serum was incubated in a water bath at 56° C. for 20 minutes; to 1 cc. of serum dilutions 1:10, 1:20 etc. to 1:5120 was added 1 cc. of a mixture of equal parts of a 2.5 per cent suspension of washed sheep cells and a 1:10 dilution of fresh guinea pig serum; the mixture was incubated at 37.5° C. for 1 hour and read directly. The titer of hemolysin was read as the greatest dilution of serum showing almost complete (more than 50 per cent) hemolysis. Many sera were tested simultaneously, known hemolytic and non-hemolytic sera being included each time. While the true "heterophile" character of the antibody thus measured has not been proven here, it may be considered as such for practical purposes. Furthermore, the results are considered only for purposes of comparison and not for their absolute values. All the sera of pneumococcus pneumonia patients were tested for agglutinins for the homologous type of pneumococcus and for several or all of the other 31 Cooper types (6).

*Subjects.* Sera were obtained during the acute disease and during convalescence from patients over 16 years of

TABLE I  
*Sources of sera tested*

Pneumococcus Type	Number of patients	Number of sera
Pneumonia patients		
I.....	42	79
II.....	4	7
III.....	13	21
IV.....	8	14
V.....	3	5
VI.....	2	3
VII.....	5	12
VIII.....	14	36
IX.....	2	2
X.....	1	1
XI.....	1	1
XII.....	3	6
XIV.....	5	10
XVIII.....	1	1
XIX.....	4	9
XX.....	3	7
XXI.....	5	6
XXIV.....	1	1
XXIX.....	1	2
XXXI.....	1	2
XXXII.....	1	2
All types.....	120	227
Fatal cases (average age 42.2 years).....	26	38
Recovered cases (average age 36.9 years):		
Tested in febrile period only.....	9	9
Tested only in postfebrile period.....	44	58
Tested before and after crisis.....	41	122
	120	227
Non-pneumonic individuals		
Controls (average age 30.4 years).....	276	276
Normal subjects after immunization.....	122	168
Total number of sera tested.....		671

age, who had definite clinical and roentgenographic evidences of lobar pneumonia and from whom pneumococci were isolated and typed. A total of 227 sera from 120 patients were tested. These included 51 sera from 24 patients treated with concentrated antibody from anti-pneumococcus horse serum. The distribution of the cases and sera in relation to pneumococcus types and to the outcome of the disease is shown in Table I.

For controls, 276 sera from as many normal adults were tested. These sera were obtained from individuals who later received injections of various pneumococcus antigens. A second group of 168 sera was obtained from 122 of the latter individuals at intervals after immunization. All of these sera were tested and found to have pneumococcus mouse protective antibody. They were also tested for hemolysin content and the results included for comparison.

## ANALYSIS OF RESULTS

*Comparison of hemolytic titers in pneumonia patients and in controls.* The frequency with which various titers of sheep cell hemolysin were encountered in the pneumonia patients, in the immunized individuals and in the normal controls is shown in Table II. The percentage incidence

TABLE II  
*Comparison of titers of sheep cell hemolysin in pneumonia patients, in immunized individuals and in normal controls*

Titer of sheep cell hemolysin (serum dilution)	Patients with pneumococcal pneumonia		Individuals immunized with pneumococcal antigens		Normal controls	
	Number of sera	Per cent	Number of sera	Per cent	Number of sera	Per cent
0†	18 <sup>2*</sup>	7.9	8	4.8	23	8.3
1:10	14 <sup>2</sup>	6.3	24	14.3	34	12.3
1:20	11 <sup>2</sup>	4.8	24	14.3	30	10.9
1:40	53 <sup>3</sup>	23.3	33	19.6	49	17.7
1:80	67 <sup>5</sup>	29.5	42	25.0	70	25.3
1:160	33 <sup>5</sup>	14.6	29	17.3	49	17.7
1:320	20 <sup>3</sup>	8.8	6	3.6	15	5.4
1:640	7 <sup>2</sup>	3.1	2	1.2	5	1.9
1:1280	4 <sup>3†</sup>	1.8	0	0	1	0.4
Number of sera.....	227 <sup>30</sup>		168		276	

\* Raised numerals represent numbers of sera obtained after horse serum administration.

† No hemolysis in 1:10 dilution.

‡ Including 1 serum with titer 1:5120.

of the various hemolytic titers among the sera of pneumonia patients is essentially similar to that found among the normal controls. This is par-

TABLE III

*Distribution of titers of sheep cell hemolysin in the sera of patients with pneumococcic pneumonia*

Titer of hemolysin (serum dilution)	Fatal cases		Recovered cases					
			Febrile period		Postfebrile period		Postfebrile (Excluding serum recipients)	
	Number of sera	Per cent	Number of sera	Per cent	Number of sera	Per cent	Number of sera	Per cent
0†	6	15.3	7	9.9	5	4.2	3	3.2
1:10	4	10.5	4†	5.6	6	5.1	5	5.4
1:20	2	5.3	2	2.8	7	5.9	5	5.4
1:40	14	36.5	18*	25.4	21	17.8	20	21.5
1:80	5	13.2	27	38.0	35	29.7	30	32.3
1:160	3	7.9	8	11.3	22	18.6	14	15.1
1:320	3†	7.9	3	4.2	14	11.9	12	12.9
1:640	0	0	2	2.8	5	4.2	3	3.2
1:1280	1†	2.6	0	0	3	2.6	1	1.1
Total number of cases	38		71		118		93	

\* Including 2 sera obtained after horse serum administration.

† No hemolysis in 1:10 dilution.

‡ Including 1 serum obtained after horse serum administration.

ticularly true when those sera are excluded which were obtained after pneumococcus antibody treatment, thus excluding the possible heterogenetic activity of the horse serum injected (7, 8). The titers of the sera of the individuals immunized

with various antigenic pneumococcus substances were also very similar in distribution to those of the controls.

The results of the tests for sheep cell hemolysin in the sera of the pneumonia patients are analyzed in further detail in Tables III, IV and V. The numbers involved in these tables are too few to be statistically significant. There seems to be a slightly higher incidence of lower titers among the sera of the fatal cases than among those that recovered (Table III). Similar differences may be noted between the sera with and those without demonstrable pneumococcus agglutinins (Table IV) and also between the sera obtained early and those obtained later (Table V). These differences are less evident if the sera obtained after horse serum administration are excluded. Differences between groups of patients, however, are difficult to evaluate because of the many factors which may come into play. The average age of the patients, for example, was greater among those who died than among those who recovered (*cf.* Table I). This may account, at least in part, for the seemingly lower titers among the former (9). A better possibility of evaluating the relationship of heterophile antibody to the course of pneumonia is offered by a study of the changes in titer of hemolysin observed in individual cases.

*Titers of sheep cell hemolysin in individual patients and changes in these titers in the course of*

TABLE IV

*Correlation of pneumococcus agglutinin titer and titer of sheep cell hemolysin in sera of patients with pneumococcic pneumonia*

Titer of homologous pneumococcus agglutinin (Serum dilution)		Titer of sheep cell hemolysin (Serum dilution)								Total number of sera	
		0	1: 10	1: 20	1: 40	1: 80	1: 160	1: 320	1: 640		1: 1280
0 {	Fatal.....	6	3	2	11	5	3	1	0	1	32
	Febrile, lived.....	4	2	2	7	21	7	2	2	0	47
	Postfebrile.....	1	4	3	6	8	6	0	0	1	29
1: 2.....		0	0	0	3	2	0	2	0	2†	9
1: 4.....		4	2	1	7*	9	8	6	1	0	38
1: 8.....		2	1*	2	8*	10	3	3*	1	0	30
1: 16.....		0	1	0	5	7	3	4	0	0	20
1: 32.....		1	1	1	4	3	3	1*	2	0	16
1: 64.....		0	0	0	2	2	0	1	1	0	6
0.....		11	9	7	24	34	16	3	2	2	108†
1: 2 to 1: 64.....		7	5	4	29	33	17	17	5	2	119‡
All sera.....		18	14	11	53	67	33	20	7	4	227

\* Including 1 serum from a fatal case.

† Including 1 with titer 1:5120.

‡ Average titer = 1:104.

§ Average titer = 1:182.

TABLE V  
Correlation of stage of the pneumonia and titer of sheep-cell hemolysin

Days after onset of pneumonia	Titer of sheep-cell hemolysin (serum dilution)									Total number of sera tested
	0	1:10	1:20	1:40	1:80	1:160	1:320	1:640	1:1280	
1-3.....	4(2)*	2(2)	1	4(1)	9(1)	4(1)	2(1)	0	0	26
4-6.....	4	5(1)	2	11(6)	18(2)	5(2)	2	2	0	49
7-9.....	4(2)	1(1)	3(1)	14(2)	11	6	1	0	0	40
10-12.....	5(2)	1	1	7	12(1)	5	4	1	2	38
13-15.....	0	4	0	6	8(1)	5	1	2	0	26
16-18.....	0	1	1	5(1)	2	2	5(1)	0	0	16
19-21.....	1	0	2(1)	1	0	3	2	0	0	9
22+.....	0	0	1	5(4)	7	3	3(1)	2	2†(1)	23
All sera.....	18(6)	14(4)	11(2)	53(14)	67(5)	33(3)	20(3)	7	4(1)	227
1-9.....	12 <sup>2</sup> †	8 <sup>3</sup>	6 <sup>2</sup>	29 <sup>2</sup>	38 <sup>4</sup>	15 <sup>1</sup>	5	2	0	115 <sup>14</sup> §
10 or more.....	6 <sup>1</sup>	6 <sup>1</sup>	5	24 <sup>5</sup>	29 <sup>3</sup>	18 <sup>4</sup>	15 <sup>3</sup>	5 <sup>2</sup>	4 <sup>3</sup> †	112 <sup>22</sup>

\* Parentheses enclose numbers of sera from fatal cases which are included.

† Includes 1 with titer of 1:5120.

‡ Superscripts represent sera obtained after specific serum treatment.

§ Average titer is 1:85; excluding those obtained after serum therapy = 1:90.

|| Average titer 1:208; excluding those obtained after serum therapy = 1:136.

*pneumonia*. In the preceding sections, the sera of pneumonia patients have been considered as a group without reference to the hemolytic titers of individual patients and to changes in these titers observed in successive sera obtained from the same individual. In Table VI are indicated the numbers of patients in whom the higher and the lower titers of hemolysin were encountered at any time. Here again, the numbers are too

few to be statistically significant. It is worth noting, however, that both the high and the low titers were frequent in each of the groups of patients. An appreciable number of the recovered patients had received injections of horse serum, thus accounting for the predominance of high titers among these patients.

It is of interest to note that 5 of the 7 fatal cases with sera which hemolyzed sheep's red

TABLE VI  
Occurrence of high and low hemolytic titers and of changes in these titers among patients with pneumococcal pneumonia

		Fatal cases	Recovered cases		
			Tested only in febrile period	Tested only during convalescence	Tested in febrile and in post-febrile period
Number of patients.....		26	9	44	41
Patients whose serum, at any time, had sheep-cell hemolysin in titers of:	(a) 1:20 or lower	9	2	10	10
		34.6	22.2	22.7	24.4
	(b) 1:160 or higher	7	1	17*	22†
		26.9	11.1	38.6	53.6
Patients in whom the hemolytic titer in successive sera showed:	(a) No change.....	6	—	6	14‡
	(b) Twofold increase.....	2	—	4	4
	(c) Twofold decrease.....	2	—	3	9
	(d) Fourfold or greater increase.....	1	—	1§	11¶
	(e) Fourfold or greater decrease.....	1	—	0	3

\* In 3 of these cases the sera were obtained after serum therapy.

† Including 9 patients with serum sickness.

‡ In 1 there was an increase and in another a decrease (twofold in each instance) before returning to original level.

§ A serum treated case.

|| In 2 instances there was a twofold drop in titer before the rise.

¶ In 5 instances there was a drop in titer preceding the rise; this drop was twofold in one, fourfold in another and twofold in the remaining 3. In 8 of these 11 patients, the increase in hemolysin was associated with serum sickness.

TABLE VII.  
Summary of observations in 5 cases referred to in the text

Name, sex and age	Pneumococcus Type	Termination of pneumonia		Patient's sera			Remarks
		Mode	Day	Day of disease obtained	Homologous type pneumococcus agglutinins (Titer = serum dilution)	Sheep cell hemolysin	
J.G. ♂ 49 years	VIII	Lysis	11-14	4	0	1 : 160	Blood culture positive 2nd to 6th day
				5	0	0	
				6	0	1 : 10	Irregular fever 20th to 39th day
				12	1 : 4	1 : 80	
				18	1 : 2	1 : 80	Subcutaneous abscess drained
				25	1 : 4	1 : 80	
				34	1 : 4	1 : 80	Well after 40th day
				39	1 : 4	1 : 80	
				52	0	1 : 1280	
W.G. ♂ 59 years	I	Crisis	8	7	0	1 : 40	Blood culture negative
				11	0	1 : 160	
				15	0	1 : 160	
E.M. ♀ 34 years	XX	Crisis	10	7	0	1 : 80	Blood culture negative
				11	1 : 16	1 : 80	
				17	1 : 4	1 : 320	
J.W. ♂ 78 years	XIV	Died	17	9	0	1 : 40	Blood cultures: 9th day positive; 17th day positive, 700 colonies per cc.
				17	0	1 : 320	
J.E.S. ♂ 15 years	II	Crisis	5	4	0	1 : 80	Blood culture negative. Concentrated anti-body (antipneumococcus horse serum) 100 cc. given on 4th and 5th day
				6	1 : 8	1 : 20	
				15	1 : 16	1 : 320	

blood corpuscles in a dilution of 1:160 or higher had a pneumococcus bacteremia which was demonstrated in the same blood. The serum of one of these patients had a hemolytic titer of 1:1280 on the day before death. This patient had Type II pneumococcus pneumonia and received serum 7 days previously or 3 days after the onset. Bacteremia persisted throughout this period. The sera of two fatal cases of pneumococcus meningitis following one month after pneumonia each had a hemolytic titer of 1:320. The meningitis was preceded in one case by mastoiditis and in the other by empyema and recurrent bacteremia. The former had a Type III pneumococcus, and the latter had Type I and received antipneumococcus serum.

Table VI also shows the number of patients in whom changes in hemolytic titer were found in successive sera. It is seen that among the fatal cases, the 12 in which multiple determinations were obtained showed no consistent changes in hemolytic titer. In 4 cases with unchanged titers and in one with an eightfold increase (J. W., see

Table VII), pneumococcus bacteremia was present and persisted until the time of death. The one patient who showed a fourfold decrease in hemolytic titer had a negative blood culture and had received injections of horse serum on the day before the second serum was obtained. The patients from whom multiple sera were obtained only during convalescence also showed no significant changes in the titers of hemolysin.

Among the 41 pneumonia patients whose sera were tested during both the febrile and the postfebrile periods, 27 or 65.9 per cent showed either no change or only a twofold difference after crisis; and decreases of this order were more frequent than increases. The 3 patients whose sera showed a fourfold decrease in titer after recovery all showed agglutinins for the homologous type of pneumococcus in the later sera, and none in the earlier sera which had the higher titers of hemolysin. Of the 11 patients whose postfebrile sera showed more than a twofold increase above the original titer, 8 had serum sickness and 2 others (W. G. and E. M., see Table VII) showed

a fourfold increase above the febrile level without having received serum. Neither of the two latter patients had demonstrable bacteremia, and one failed to exhibit agglutinins for the homologous Type I pneumococcus in the postfebrile serum.

One patient (J. G., see Table VII) who showed a marked increase in the hemolytic action of his serum is of some interest. When first tested the titer of his serum was 1:160. This declined in the next 2 days. Blood cultures during this time yielded Type VIII pneumococcus repeatedly. One week later, after recovery by lysis, the hemolytic titer was 1:80, at which level it remained for 4 weeks. During this interval the patient had low grade fever and several subcutaneous abscesses. These were drained and yielded Type VIII pneumococci in pure culture. Two weeks after all signs of persistent infection had cleared, the hemolytic titer of the serum was 1:1280. This is the only case in which a significant drop in titer occurred during the bacteremic period to be followed by a sharp rise after complete recovery.

Other observations of interest may be noted. In Table VII are noted the relevant data in 4 patients to whom reference has been made in the preceding paragraphs. A fifth patient (J. E. S.) with Type II pneumonia is also included. This patient showed a significant decline in hemolytic titer following serum therapy to be followed later by an increase above the original level associated with the clinical picture of serum sickness. This decrease was the largest observed after serum administration. In 3 other patients twofold decreases were noted within the first day after administration of the therapeutic sera. The average titer of hemolysin in the first 9 days of the disease was 1:90 when horse serum had not been given and 1:45 in those sera obtained after therapeutic antibody injections. After the ninth day the average titer of the sera of cases treated with antipneumococcus horse serum was 1:500 as compared with 1:136 in those not so treated.

The highest titer of hemolysin noted was in a dilution of 1:5120 in serum obtained from a negro of 17 with Type I pneumonia 3 weeks after he had had symptoms of serum sickness. The hemolytic titer during his serum sickness was 1:1280.

Although mild serum sickness was frequent

among the antibody treated cases, immediate reactions were not observed. Only one patient, among those studied, had a chill following a second dose of serum (13 cc.). No reaction followed the first dose of 2 cc. in this patient. The hemolytic titer of this patient's serum was 1:320 before antibody treatment was begun.

There was no correlation whatever between hemolytic titer and the type of pneumococcus.

#### DISCUSSION

The present study was undertaken to determine whether the pneumococcus, in causing pneumonia in man, also acts as a heterophile antigen and, if so, whether this is of significance in relation to the course of the disease. The possibility that it may do so was suggested by the heterogenetic activity of various pneumococcus strains when they were injected into rabbits or when they produced experimental infections in this species (1, 2, 3, 4). The protective (1) and curative (4) value of heterophile antibody, when actively or passively induced in rabbits, also suggested that such antibody might have therapeutic significance in human cases of pneumonia.

The weight of the evidence adduced from a study of the hemolytic action of sera of pneumonia patients on sheeps' red blood corpuscles indicates that such a heterophilic activity, if present, is low grade, infrequent and probably of no importance in relation to the course and outcome of the disease. 1. During the febrile period in the favorable cases of pneumonia the titers of sheep cell hemolysin in the serum are the same as in normal controls. 2. Moderately high titers of sheep cell hemolysin are frequently encountered in the serum of patients with pneumococcus bacteremia, even shortly before death. 3. Bacteremia may be present without altering the hemolytic titer, and occasionally the hemolytic titer may be found to increase in spite of an increasing bacterial invasion. 4. Low titers are encountered frequently in patients who recover. 5. Successive sera obtained during the febrile and postfebrile period show that the titers of hemolysin may drop just as frequently and to the same degree as they may rise in the course of convalescence from pneumonia. 6. Significant increases in hemolytic titer are en-

countered only rarely, except following the administration of antipneumococcus horse serum.

On the other hand, the slightly higher incidence of lower titers among the sera of fatal cases and the somewhat greater frequency of higher titers during convalescence might possibly be considered to result from the heterogenetic action of pneumococci. The lower titers in the fatal cases might be the result of partial absorption of the "natural" heterophile antibody by the heterophile component of the pneumococcus antigen, and the higher titers would then result from the production of antibody by this antigen. This possibility is not borne out by the consideration of the titers of hemolysin in the sera of individual patients and by the changes noted when successive sera from the same patient are tested.

The results of the tests for hemolysin in immunized individuals are also of interest. The antigens used exhibited considerable species activity as indicated by the demonstration, in the recipients, of antibodies for types of pneumococci other than those from which they were prepared (13). The hemolysin titers in the sera of individuals immunized with these antigens were the same as in the normal controls. The heterophile component of the pneumococcus which is active in animals is also species specific, but since its exact composition is not known, beyond its association with the lipoid fraction, the bearing of the present findings on this phase of the problem can not be evaluated.

These observations are not entirely incompatible with the results of the rabbit experiments previously mentioned. The optimal heterogenetic activity of pneumococci was obtained after vigorous treatment of the organisms, for example by boiling, before they were given to the animals. Furthermore, the strain which exhibited the greatest heterogenetic activity was a completely avirulent one originally derived from a Type I pneumococcus. Another very active Type I strain was relatively avirulent as indicated by the marked increase in the fatality rate among rabbits with dermal pneumonia produced by this organism after 2 and especially after 33 rabbit passages. When this latter strain was used to infect rabbits after 33 rabbit passages, no protection was afforded by heterophile antibody actively or pas-

sively induced in the animals. It is possible, therefore, that the heterogenetic property of pneumococci is related to changes which occur during cultivation and in the methods of treating the organisms, and has no particular bearing in relation to actual infection. In such infections as pneumonia which are associated with highly type specific and usually virulent strains, the heterogenetic properties of pneumococci probably have no significance.

In contrast to the unconvincing evidence presented by these findings, it is of interest to consider the more positive observations in the patients who received concentrated antipneumococcus horse serum therapeutically. The value of this homologous serum in the treatment of Type I pneumococcus pneumonia is now well established. That horse serum acts as a heterophile antigen in man has been shown by many workers (7, 8, 10, 11, 12). That this refined fraction is potent as a heterophile antigen is indicated by the observations presented, namely, by the decrease in titer of sheep cell hemolysin in the first few days after serum administration and the marked increase in this titer in the same patients later, particularly when serum sickness occurs. There is no indication, however, that this heterophile activity has any influence on the course of the pneumonia. Favorable effects were observed from the use of serum in spite of an average decrease of 50 per cent in the hemolytic titer of the serum. Fatal outcome, on the other hand, has been observed even after the heterophile antibody had increased significantly in patients treated with serum, presumably in response to the horse serum injections and in spite of persistent pneumococcus invasion. Even the immediate reactions to injections of horse serum, such as were observed by Davidsohn (10) with crude therapeutic antisera in patients with heterophile antibody, were not encountered after the intravenous injections of the refined antipneumococcus concentrates. These observations indicate that even known heterophile antigen has no particular relationship to the course of pneumonia in man.

On the basis of all of the findings here presented, it is felt that the heterophile activity of the pneumococcus is probably of no significance in human cases of lobar pneumonia.



## SUMMARY AND CONCLUSIONS

1. The content of hemolysin for sheep red blood corpuscles was measured in 227 sera from 120 patients with lobar pneumonia due to various types of pneumococci. Among these, 51 sera were from 24 patients treated with concentrated anti-pneumococcus horse serum. Similar tests were made on the sera of 276 normal individuals and in 168 sera from 122 persons who received immunizing injections of pneumococcus antigens.

2. The frequency of various titers of sheep cell hemolysin among the sera of pneumonia cases, particularly when those obtained after administration of therapeutic serum were excluded, was the same as in normal subjects, and this was also true of the sera of the immunized individuals.

3. Successive sera from the same patients only rarely showed significant increases in the titer of sheep cell hemolysin except in relation to horse serum injections.

4. There was no constant relationship between the hemolytic titer of the serum and the outcome of the disease. High titers and increasing titers were found in sera from fatal cases, even in the presence of bacteremia. Low and declining titers were frequently encountered coincident with normal recovery.

5. Concentrated pneumococcus antibody (Felton) is an active heterophile antigen. This is shown by the decrease in hemolytic titer for a few days after its administration and the subsequent marked rise in this titer in a considerable proportion of cases. The heterophile activity of this antibody, however, has no relation either to the occurrence of immediate untoward reactions or to the course and outcome of the pneumonia.

6. Heterophile antibody probably has no significance in human cases of lobar pneumonia.

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# THE METABOLIC EFFECTS OF HUMAN THYROGLOBULIN AND ITS PROTEOLYTIC CLEAVAGE PRODUCTS

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In previous communications, (1, 2, 3, 4, 5) it has been pointed out that two important questions with regard to the nature of thyroid colloid must be answered because they bear upon the normal and pathological physiology of the gland. The first of these is: Does the colloid of "toxic" glands show significant differences from normal or "non-toxic" colloid? The second question is: Does the activity of the thyroid secretion depend solely upon its thyroxin content? This paper includes both chemical analyses and biological assays so correlated as to bring out possible causal relationships to the metabolic effects produced. As explained elsewhere, (1) a routine standard procedure was adopted for administering thyroid material to patients with myxedema. The magnitude of the metabolic response could then be used as a gauge of the potency of the preparation or drug administered.

The thyroglobulins were prepared by extraction of freshly iced, surgically excised glands with 0.02 normal sodium hydroxide solution. The protein was subsequently precipitated isoelectrically; and then redissolved and reprecipitated repeatedly. The preparation of the di-iodotyrosine peptone and thyroxin peptone digests was accomplished as described by Harington and Salter (6). Undigested protein was eliminated by heat coagulation and by the acetone treatment described by these authors. Total iodine was estimated according to the procedure of Kendall (7). The thyroxin moiety was estimated by the procedure of Harington and Randall (8) and checked by the methods of Leland and Foster (9) or of Blau (10).

The response of patients to test fractions of thyroid material was judged by comparison with the standard curve of reference obtained by administering natural thyroxin polypeptide (1). When this substance was fed in solution in daily doses containing 0.5 mgm. iodine, the daily rise

in basal metabolic rate was found to average about 2.5 points (1). By this method the response of myxedematous patients could be calibrated when test preparations were administered. The reliability of this procedure has been discussed in a previous communication (5). A patient was never used for more than one assay, unless the first result was completely negative.

## *Effect of thyroglobulins upon basal metabolic rate*

**Colloid goiter.** Two adult patients with typical spontaneous myxedema were treated with thyroglobulin prepared from human multiple colloid adenomatous goiters, excised surgically. The daily dose, by mouth, was 0.5 mgm. in terms of total iodine. The responses produced (recorded in Figure 1, Curve A and B) corresponded to the standard response obtained from an equivalent amount of thyroxin polypeptide, based upon the total iodine content. One cretin, aged twenty-one, previously untreated, was given 0.5 mgm. of iodine in the form of this same thyroglobulin. The result (shown in Figure 1, Curve C) was likewise of the same order of magnitude as the standard response. The average daily rise in basal metabolic rate was 2.8 points per day, as against the standard rise (1) of 2.5 points per day.

Chemical analysis by the method of Harington and Randall (8) showed 30 per cent of the iodine in this preparation to exist apparently as thyroxin. Nevertheless, the response indicates that 100 per cent of the iodine is active. This confirms work previously published to the effect that the activity of thyroid hormone is determined not by its thyroxin iodine, but by its total organic iodine.

**Exophthalmic goiter.** Three other patients were treated with thyroglobulin prepared from glands surgically excised from patients with hyperthyroidism, showing hyperplastic glands. Chemical analysis by the method of Harington and

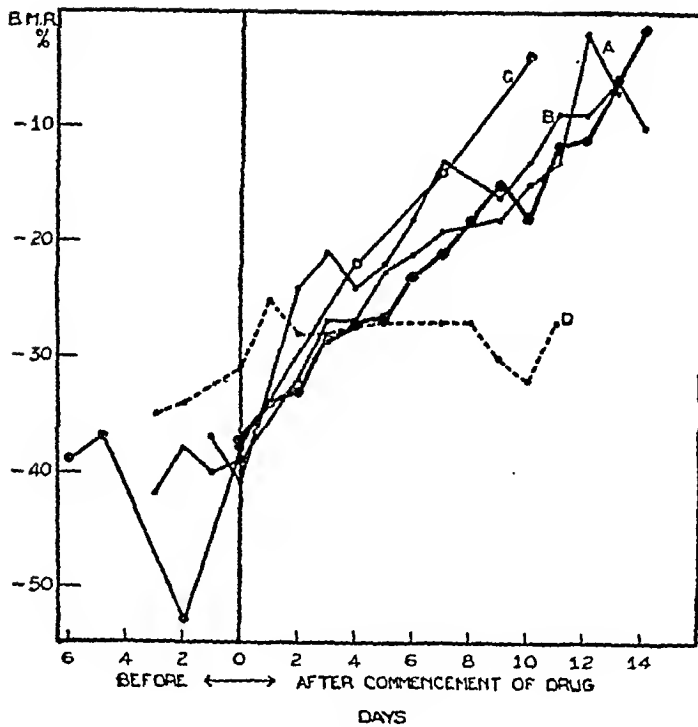


FIG. 1.

Curves *A* and *B* represent the calorigenic responses of two patients with spontaneous myxedema who received daily oral doses of thyroglobulin (containing 0.5 mgm. of total iodine) derived from human, multiple colloid adenomatous goiters. Curve *C* represents the calorigenic response of a cretin to this same form of thyroglobulin. Curve *D* represents the calorigenic response of one patient with spontaneous myxedema to the daily oral administration of 0.5 mgm. of iodine in the form of the thyroglobulin obtained from a colloid gland removed in an endemic goiter region. The heavy solid line, present in all the charts, is the standard response to daily doses of 1.0 mgm. of natural thyroxine polypeptide (0.5 mgm. total iodine).

Randall (8) showed 25 per cent of the iodine in this preparation to exist apparently as thyroxine. By the method of Leland and Foster (9) the value was 20 per cent. Two of the patients had spontaneous myxedema; the third had cachexia strumipriva, complicated by rheumatic heart disease (without myocardial failure). The responses of the three patients insofar as average slope is concerned were altogether similar to those of patients receiving non-toxic colloid thyroglobulin, the average daily rise in metabolism being in the case of "toxic" thyroglobulin 2.9 points per day, and in that of "non-toxic" thyroglobulin 2.8 points per day. It is true that inspection of the three curves for "toxic" thyroglobulin shown in Figure 2 reveals a hump in each about the fifth to sixth day. We have observed similar humps

in isolated instances in the past and have not discovered that they have any significance.

In the interpretation of these results, it must be remembered that the source of thyroglobulin was tissue excised from patients who had received iodine therapy preliminary to operation.

*Simple colloid goiter.* In addition, to one patient with spontaneous myxedema there was given 0.5 mgm. of iodine daily in the form of thyroglobulin obtained from a large colloid gland removed from a patient living in an endemic goiter region.<sup>1</sup> As shown in Figure 1, Curve *D*, there followed no significant metabolic response. This material was found on chemical analysis to contain only 0.006 per cent of total iodine, and of this only 5 per cent (relative percentage) was apparently in the form of thyroxine by the analytical method of Harington and Randall (8). The

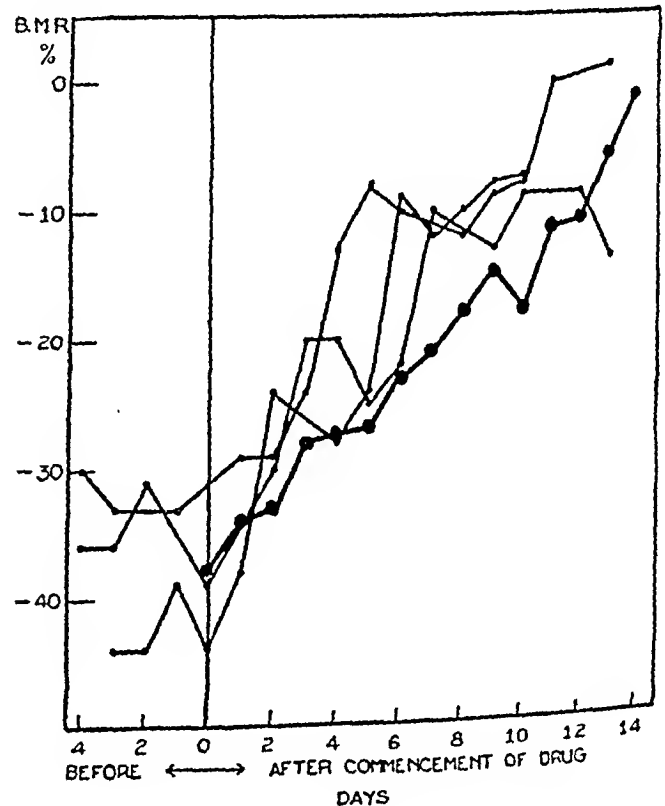


FIG. 2. THE RESPONSE OF THREE PATIENTS WITH MYXEDEMA, TWO SPONTANEOUS AND ONE CACHEXIA STRUMIPRIVA, TO THE DAILY ORAL ADMINISTRATION OF THYROGLOBULIN DERIVED FROM HUMAN HYPERPLASTIC ("TOXIC") GLANDS, EACH DOSE CONTAINING 0.5 MGm. TOTAL IODINE (LIGHT SOLID LINES AND SOLID CIRCLES).

The heavy solid line is the standard curve, as in Figure 1.

<sup>1</sup> We are indebted to Professor Frederick A. Collier of the University of Michigan for this material.

check value obtained by Blau's method (10) was per cent.

### *Action of peptones of human thyroglobulin*

When thyroglobulin is digested with pepsin under appropriate conditions, a considerable portion of the organically-bound iodine remains insoluble in dilute acid at pH 5.0 (6). This insoluble fraction is variable in amount, but often amounts to one-third of the total iodine. It consists partly in (a) undigested protein and partly in (b) peptones containing thyroxine as the chief source of iodine. Harington and Salter (6) found that after such isoelectric precipitation of the digest, the iodine-containing peptones which remained in solution yielded no thyroxine. This second iodopeptone was shown by Harington and Randall (11) to yield di-iodotyrosine<sup>2</sup>.

**Thyroxine peptone.** Three patients with spontaneous myxedema were treated with thyroxine peptone, administered by mouth in daily doses containing 0.5 mgm. iodine. One of these failed to respond (Curve A, Figure 3), and in this case the material was administered suspended in water, but undissolved. In the other two cases, the peptone was dissolved in dilute alkali before administration, and both these patients made significant responses (Curve B and C, Figure 3), although in one of them (Curve B) it was somewhat sub-standard. In the latter case, however, subsequent daily administrations of thyroglobulin (from colloid adenomatous glands) for six days failed to produce any additional rise. The inference, therefore, is that this patient had obtained a maximal response, even though sub-standard. Of these two effective peptone preparations, one (Curve B, Figure 3) was made from multiple colloid, non-toxic adenomatous goiter, and the other (Curve C, Figure 3) from toxic diffusely hyperplastic goiter.

**Di-iodotyrosine peptone.** Three patients with spontaneous myxedema were treated with di-iodotyrosine peptone in daily oral doses containing 0.5 mgm. of iodine. None of these patients made

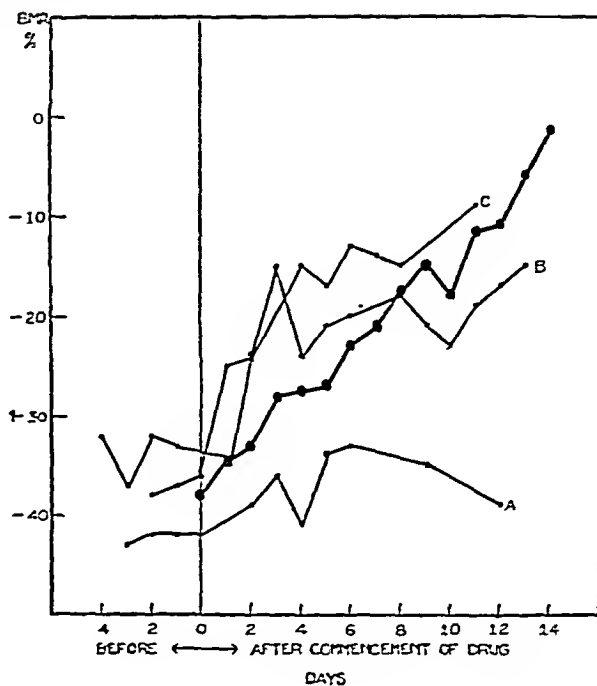


FIG. 3. THE RESPONSE OF THREE PATIENTS WITH SPONTANEOUS MYXEDEMA TO THE ADMINISTRATION OF THYROXINE PEPTONE IN DAILY ORAL DOSES CONTAINING 0.5 MGm. TOTAL IODINE.

The patient represented by Curve A received undissolved material. Curve B represents the response to thyroxine peptone derived from non-toxic colloid adenomatous goiter and Curve C to peptone derived from hyperplastic "toxic" goiter. The heavy solid line is the same as in Figure 1.

an unequivocal response. All three patients subsequently made a good response to an active thyroid preparation. The conclusion is to be drawn, therefore, that thyroxine peptone possesses standard activity, but that di-iodotyrosine peptone in standard doses is inert.<sup>3</sup>

### *Effect of synthetic (racemic) glycyl-thyroxine*

Through the kindness of Professor C. R. Harington, we were able to test the activity of a synthetic dipeptide made in his laboratory and described by Ashley (12). This material was administered intravenously to two patients with spontaneous myxedema in daily doses corre-

<sup>2</sup> The thyroxine-containing peptone can be partially purified by re-precipitation and by treatment with acid acetone (6). The di-iodotyrosine peptone can be essentially freed from inorganic iodide by concentration of the acid solution under reduced pressure.

<sup>3</sup> It is necessary to note, however, that in assays in two other cases, in which three and six times the standard iodine dosage (0.5 mgm.) of di-iodotyrosine peptone was given, definite responses did occur. These data will be the subject of a later communication.



PROCEEDINGS OF THE TWENTY-SEVENTH ANNUAL MEETING OF THE  
AMERICAN SOCIETY FOR CLINICAL INVESTIGATION  
HELD IN ATLANTIC CITY, N. J., MAY 6, 1935

READ BEFORE SECTION A

*Physiologic Effects of the Inhalation of Helium with Oxygen on the Mechanics of Respiration.* By ALVAN L. BARACH, New York, N. Y.

The low density of helium was made use of to provide a respirable gas consisting of 20 per cent oxygen and 80 per cent helium which was one-third as heavy as a comparable air mixture. When prolonged respiratory obstruction was produced in dogs by breathing through narrowed orifices, the effect of helium-oxygen mixture in comparison to air was shown by marked decreases in the inspiratory and expiratory pressures and in the pressure in the pulmonary airways. The latter measurement was obtained by connecting a water manometer to a tube leading from the dog's mouth to a closed respiratory apparatus.

After three hours of breathing air through a narrowed orifice, dogs showed striking increases in intrapleural pressures, marked reduction in the pressure in the pulmonary airways and a progressively diminished tidal volume. The effect of helium with oxygen at this stage was to reduce the intrapleural pressures, increase the tidal air and to increase the external pulmonary pressure. A stage was produced when a dog could not inhale air but could take in a comparable helium-oxygen mixture.

*Studies on the Volume Flow of Blood in the Hands of Cases of Peripheral Vascular Disease.* By NORMAN E. FREEMAN (introduced by A. V. Bock), Boston, Mass.

The rate of blood flow through the hand, with the temperature controlled, measured by a modification of the plethysmographic technique of Hewlett and Van Zwallowen has been studied. An increase in the volume-flow of blood was found to result from raising the local temperature. The curve obtained under basal fasting conditions was characteristic for the patient from day to day. Immediately after sympathectomy the rate of flow was constant and did not vary with changes in local temperature. Six months later the circulation again was found to change with the local temperature. The logarithm of the rate of flow was a linear function of the reciprocal of the absolute temperature. After release of a circulatory occlusion the excess blood flow above the basal level within certain limits exactly accounted for the amount of deprivation. It is therefore suggested that in the normal hand the circulation is modified through the sympathetic nervous system by the thermal-regulatory needs of the body. After removal of the sympathetic nerves, the blood flow is controlled by the metabolic requirements of the tissues.

In the treatment of Raynaud's disease, active vasodilatation through the calorogenic action of dinitrophenol has proven of value.

In obliterative vascular disease, the power of reflex vasodilatation is lost. Application of local heat increases the discrepancy between metabolic needs and blood supply.

*Residual Vasoconstriction from Medulli-adrenal Secretion after Cervicothoracic and After Lumbar Ganglionectomy: An Explanation of the Different Results after Sympathetic Denervation of the Upper and Lower Extremities in Raynaud's Disease.* By JAMES C. WHITE, Boston, Mass.

Elliott (1905) first called attention to the fact that the contractile mechanism in the smooth muscle cell becomes abnormally sensitive to adrenaline after its postganglionic neurones have degenerated. Other manifestations of this phenomenon have been demonstrated by the work of Meltzer and Auer (1904), of Hartman, McCordock, and Loder (1923), and of Cannon, Lewis, and Britton (1926). The importance of this effect in man as a cause of failure after sympathetic ganglionectomy for Raynaud's Disease has recently been demonstrated by Smithwick, Freeman, and White (1934). In a second article we showed that this chemical mediation accounts for the ability of the arteries in the rabbit's ear to constrict during insulin hypoglycaemia after the postganglionic neurones have degenerated, and further that this effect is inhibited by inactivation of the adrenal glands.

With the assistance of two Harvard Medical students, I have continued this investigation during the past fifteen months. We have shown that:

1. The residual vasomotor changes in the rabbit's ear, after degeneration of the postganglionic neurones by resection of the superior cervical sympathetic ganglion and division of the remaining vasomotor neurones which run with the sensory nerves (described by Grant, Bland, and Camp, 1932), are in great part due to circulating adrenaline and can be largely eliminated by adrenal inactivation (shown by photographs of ear vessels).

2. On the other hand, when the preganglionic neurones are interrupted by stellate ganglionectomy or by division of the upper four anterior thoracic roots, the vessels in the rabbit's ear remain dilated—neither cold, pain, emotion, intravenous adrenaline, nor the adrenaline secreted during insulin hypoglycaemia cause any striking degree of vasoconstriction (also shown by photographs of ear vessels).

A comparison of these two experimental procedures reveals in a striking manner the superior degree of vasodilation which is maintained after the preganglionic fibres are cut. This operation interrupts all central efferent vasoconstrictor impulses, but renders the smooth muscle of the arterioles but very slightly sensitive to circulating medulli-adrenal secretion.

I wish to propose this fundamental difference in reaction as the explanation in man of the frequent poor results after cervicothoracic ganglionectomy and the constant satisfactory results after the lumbar operation. Anatomically the inferior cervical, and first and second thoracic ganglia are known to contain the trophic cells of the great majority of postganglionic neurones to the brachial plexus. On the other hand, as far as the foot is concerned, the lumbar ganglia for the most part contain descending preganglionic fibres. The great majority of postganglionic neurones to the sciatic nerve originate in the three upper sacral ganglia and remain intact after the lumbar operation. Further confirmation of this is given by the skin temperature response to intravenous adrenaline after all four extremities have been denervated (charts). These cases show striking vasospasm in the hands, but only a slight drop in the skin temperature of the toes (temperature charts). We have observed spontaneous vasospasm in the hands on emotional disturbances within ten days after sympathectomy, but no clinical evidence of this in the feet. I believe that sensitization of the denervated smooth muscle in the digital arterioles to adrenaline is a better explanation of this phenomenon than local sensitivity to cold (Lewis, 1930), incomplete sympathectomy (Adson, and Leriche and Fontaine, 1933), or the common argument that Raynaud's disease is more severe in the hands than in the feet.

*The Significance of Latent Scurvy as an Etiologic Factor in Rheumatic Fever.* By MARK P. SCHULTZ (by invitation), JULES SENDROY (by invitation), and HOMER F. SWIFT, New York, N. Y.

Basing the plan upon confirmation of Rinehart and Mettier's observation that infection of scorbutic guinea pigs with Group C streptococci induced cardiac lesions somewhat resembling those of rheumatic fever, and that only a mild degree of scurvy was requisite, the following course was pursued.

The etiologic relationship of relative vitamin C deprivation to the onset and recurrence of rheumatic fever was studied clinically. By estimating ascorbic acid excretion quantitatively in individuals under controlled intake a method was evolved for testing the relative degree of saturation with vitamin C. The vitamin C excretion level was compared with the capillary permeability in patients with rheumatic fever shortly after admission to the hospital and for periods after prolonged high ascorbic acid ingestion.

A second group of rheumatic children, some with active disease, were examined periodically for several months, both with ordinary clinical technique and with testing of capillary permeability. An attempt was made to judge the influence of various habitual diets in respect of vitamin C intake with the tendency to develop rheumatic fever; and one sub-group received large daily doses of ascorbic acid to determine whether this would influence the appearance of relapses.

The results indicate that the occurrence and persistence

of rheumatic fever is not exclusively conditioned by the existence of latent scurvy.

*Studies of Vitamin C Excretion and Saturation.* By JOHN B. YOUNG and (by invitation) MARVIN B. CORLETTE, JOSEPH H. AKEROYD and HELEN FRANK, Nashville, Tenn.

The daily excretion of vitamin C in the urine and the degree of retention following the administration of test doses has been studied in a group of presumably normal subjects (hospital staff and personnel) on a diet which was considered to be well balanced and adequate. Similar studies were made on subjects whose diets were suspected of being inadequate in various respects but representative of the diets of a large group of people in this locality. Most of the normal subjects were found to excrete between 15 and 30 mgms. of vitamin C daily, amounts which are believed to fall within the range of normal. In them the amount of retention following the administration of test doses indicated a normal degree of saturation. A few supposedly normal subjects excreted less than 15 mgms. per day and on test doses showed an apparently significant degree of unsaturation. Many of the subjects whose diets were suspected of being inadequate were found to excrete small amounts of the vitamin.

*Relation of Sunlight to Lesions of Pellagra.* By JULIAN S. RUFFIN (by invitation) and DAVID T. SMITH, Durham, N. C.

In an analysis of the symptoms in 108 cases of pellagra it was found that the general symptoms were initiated or increased by the development of the dermatitis. Sore mouth or tongue was present in sixteen patients before the development of the dermatitis and in seventy-five after the dermatitis occurred. Diarrhea was present in eight instances before and fifty-two instances after development of dermatitis. Mental disturbances were not present in any of the cases before the dermatitis occurred, but developed co-incidental with or after it in twenty patients.

Unilateral skin lesions were produced by unilateral exposure to sunlight in eleven out of twenty-five patients. None of the fourteen patients who failed to get dermatitis on re-exposure to sunlight developed sore tongue, diarrhea or dementia.

In the eleven patients who did develop dermatitis the stools increased from an average of two per day for the three days prior to exposure to an average of five for the three days immediately after exposure. On re-exposure after treatment neither dermatitis nor diarrhea developed.

Sore tongue was increased in eight out of eleven patients who developed dermatitis after exposure to sunlight. Re-exposure after treatment produced neither sore tongue nor dermatitis.

Dementia occurred in three out of eleven patients who developed dermatitis after exposure to sunlight. Two patients died. One who recovered with treatment

was subsequently exposed to sunlight without any symptoms developing.

Unilateral skin lesions have been produced in pellagra by unilateral exposure. When dermatitis occurs as a result of exposure there is a tendency for the patient to develop diarrhea, sore tongue and sometimes dementia. These symptoms do not occur in patients when they have been exposed to sunlight after receiving adequate treatment.

In evaluating the results of treatment with any specific substance it should be remembered that a considerable number of patients with the classical symptoms of pellagra will recover while on a "G" deficient diet without treatment.

*The Prevention of Pellagra by Means of Parenteral Liver Extract Administration.* By AUSTIN B. CHINN (by invitation) and TOM DOUGLAS SPIES, Cleveland, O.

During the past five years it has been demonstrated in the Medical Clinic of the Lakeside Hospital that the administration of liver extract in repeated large doses is very effective in the treatment of pellagra. Since many of these pellagrins returned to their original environment after leaving the hospital and again imbibed heavily of whiskey, they soon lost desire for food and subsequently pellagra returned. In view of this fact, it seemed worthwhile to determine whether the recurrence of pellagra could be prevented in these individuals in whom it had occurred most frequently. Accordingly, 4 individuals who were among those most frequently admitted to the hospital with pellagra were selected as suitable cases for study. In each instance injection of liver extract was begun immediately after an attack of the disease and was continued for 1 year. (The patients remained in their natural environmental conditions and continued to drink large amounts of whiskey during this period.)

The 4 patients treated as outlined above had a total of 17 hospital admissions during the 9 months just preceding the beginning of this experiment. In contrast, following the beginning of the injections, none of the 4 patients was admitted to the hospital with pellagra during the subsequent 12 months. Interpretation of these observations suggests that the frequent administration of large doses of liver extract aids in the prevention of pellagra.

*The Neutralization of Encephalitis Virus (St. Louis, 1933) by Serum.* By R. S. MUCKENFUSS, J. E. SMADEL (by invitation) and ELIZABETH MOORE (by invitation), St. Louis, Mo.

The neutralization test as used with the virus of encephalitis (St. Louis, 1933) depends on adding serum to virus diluted close to the limit of infectivity. As this is done, errors resulting from chance variations in the amount of virus inoculated occur, which reduce the significance of differences in the number of mice dying in individual tests. These factors have been studied and

the strain of mice, the strain of virus, and the number of mice used taken into consideration. Virus was neutralized by the serum of over 80 per cent of individuals convalescent from encephalitis in 1933 as well as by the serum of a number of individuals residing in the area. The sera of several individuals recovered from different types of encephalitis in France have been received and none of these neutralized the virus. The significance of these findings is discussed.

*Studies on a Filterable Virus Recovered from Cases of Clinical Influenza in Man.* By THOMAS FRANCIS, JR., and (by invitation) T. P. MAGILL, New York, N. Y.

A filterable virus was recovered from the pharyngeal washings of influenza patients in Puerto Rico, New York and Philadelphia. The inoculation of the virus into the nasal passages of anaesthetized ferrets or mice produces an experimental disease characterized by a bluish-red edematous consolidation of the lung. In ferrets the disease is usually non-fatal, while most of the infected mice die.

The serum of recovered ferrets mixed with virus and inoculated into the nasal passages of mice prevents the development of the pulmonary lesions, while normal ferret serum has no effect. It has been found that the sera of ferrets recovered from infection with either the Puerto Rico or the Philadelphia strains reciprocally neutralize both strains of virus. In addition, the serum of a horse immunized by Andrewes, Laidlaw and Smith against the British (W. S.) strain of human influenza virus also neutralizes both the Puerto Rico and Philadelphia strains. The serum of swine recovered from infection with swine influenza (obtained from Dr. Shope) fails to neutralize these two human strains of virus. These results indicate that the strains of influenza virus recovered from patients in different parts of the world are immunologically identical, while that of swine influenza differs serologically.

Additional evidence of the identity of the Puerto Rico and Philadelphia strains of virus was obtained by immunization experiments. White mice were vaccinated subcutaneously with living virus obtained from mice or ferrets infected with the Puerto Rico strain of virus. They were then found to be immune to the homologous strain of virus when tested by the intranasal route. When retested with the Philadelphia strain of virus, they were found to be actively immune to this, the heterologous strain, as well.

Furthermore, the serum of influenza patients in New York, taken from the same individuals during the acute and convalescent stages of the disease, was tested against the Puerto Rico strain of virus. The convalescent serum uniformly protected mice, while the serum during the acute illness did not. Control tests were made with the serum of patients in the acute and convalescent stages of lobar pneumonia. In none of these cases did the effect of the convalescent serum on the virus differ from that of the serum taken during the acute stage of pneumonia.



These results clearly indicate that the virus is causally related to human influenza.

*Immunization of Human Subjects with the Specific Soluble Substance of Type VIII Pneumococcus.* By MAXWELL FINLAND and (by invitation) JAMES M. RUEGSEGER, Boston, Mass.

The carbohydrate used was prepared from Type VIII pneumococcus by Dr. Rachel Brown. This material is readily soluble in water or physiologic saline and precipitates the homologous antiserum in a dilution of 1:4,000,000. It shows marked cross precipitation reactions with Type III SSS. Dr. Brown's chemical analysis of this material is as follows: nitrogen 0.75 per cent; phosphorus 0.11 per cent; moisture, 5.78 per cent; and ash 6.37 per cent. She has observed that this material produces purpura in mice.

This carbohydrate was given to hospital patients, who had no history of recent pneumonia or other febrile illness. It was given in doses of 0.01 mgm. intracutaneously or 1.0 mgm. subcutaneously. Studies of the blood were made before and at intervals after the injection for agglutinins and protective antibodies of the serum and for the phagocytic action of defibrinated blood. Type III pneumococci and, in some cases, also Type I pneumococci were used as controls. Each subject developed type specific antibodies, the response being quantitatively similar to that following the recovery from Type VIII pneumococcus pneumonia. The response following the larger dosage did not differ significantly from that following the small intracutaneous injections. No appreciable amount of antibody against Type III, or of Type I, could be demonstrated.

In another group similar experiments were conducted using the Type III carbohydrate. In the subjects who showed a response, this was type-specific and no appreciable amount of Type VIII antibodies developed.

*A Correlation of the in vitro Thermal Death Time of the Gonococcus with the Duration of the Artificial Fever in the Treatment of Gonococcal Infections.* By S. L. WARREN, R. A. BOAK (by invitation), and C. M. CARPENTER (by invitation), Rochester, N. Y.

The *in vitro* thermal death time has been determined at 41.5° C. on 95 strains of the gonococcus and found to vary between 6 and 27 hours. Individual patients may harbor several strains differing in their resistance to heat. Artificial fever of from 5 to 17 hours at 41.5° C. has been given to 118 patients: 64 females, 54 males. Of this number 60 per cent have been clinically and bacteriologically negative for from 3 months to 3 years, while an additional 20 per cent have been negative for from 1 to 3 months.

Each of 11 patients was subjected to a single fever at 41.5° C., equal in length to the thermal death time of his culture, following which there was an immediate subsidence of all clinical symptoms and a bacteriological "cure." Similar results were obtained in 9 patients when the fever period was  $\frac{1}{4}$  to  $\frac{3}{4}$  of the thermal death time, suggesting the assistance of defense factors in the

body. Failure in those cases in which the fever was shorter than the thermal death time may be due to the inability of the host to supply the necessary supplemental resistance where the bactericidal effect of the fever has been inadequate.

*The Cardiac Output and the Work of the Heart in Hypothyroidism.* By MARK D. ALTSCHULE (introduced by Herrman L. Blumgart), Boston, Mass.

The cardiac output was estimated in patients with angina pectoris and congestive heart failure at various levels of metabolism before and after total thyroidectomy. Both the acetylene and the ethyl iodide methods were used. They were found to yield identical results. Following operation, when the basal metabolic rate was approximately minus 30 per cent, the cardiac index was decreased to about 1.3, a fall of about forty per cent. This disproportionate decrease in cardiac output was associated with an increase in arteriovenous oxygen difference.

The velocity of blood flow was measured in the same patients by the decholin method. With rare exceptions, changes in the cardiac output were accurately reflected in the velocity of blood flow.

The left ventricular work was calculated by means of the formula of Evans ( $W = QV + mV^2/2g$ ). The work of the heart was found to parallel the cardiac output. A fall in metabolism of thirty per cent was accompanied by a diminution in the work of the heart of forty per cent, i.e., the work of the heart was decreased more than one would expect merely from the fall in basal metabolism. These observations are of importance in explaining the relief obtained in heart disease following total thyroidectomy.

*The Action of Drugs on Myocardial Function in Cardiac and Circulatory Disease.* By ISAAC STARR, JR., and (by invitation) J. S. DONAL, A. MARGOLIES and C. J. GAMBLE, Philadelphia, Pa.

This investigation comprised about 250 estimations of cardiac output on 50 patients in the basal condition.

The rapidly acting drugs (morphine, adrenalin, ephedrine, quinidine, pitressin, choline derivatives and nitrates) were studied as follows. An orthodiagram and electrocardiogram were secured. Duplicate estimations of cardiac output and metabolism, and repeated determinations of blood pressure and pulse rate were made with the subject lying at rest. A drug was then administered and, when its action had manifested itself, the study was repeated.

Digitalis was studied by a repetition of these estimations on different days, together with determinations of vital capacity, venous pressure and circulation time.

Accepting Starling's Law of the Heart as the expression of normal cardiac function, myocardial stimulation may be defined as that condition which permits the heart to do more work per beat in proportion to its size; and depression as the reverse.

Such a conception resolves the discrepancy alleged to exist between the action of digitalis in the clinic and in

animal experiments. In our cases digitalis causes myocardial stimulation almost without exception. Adrenalin and ephedrine are likewise stimulants. Quinidine and pitressin have little depressant action in therapeutic doses.

Two patients have been studied during angina pectoris and after relief by nitrites.

*Effect on Cardiac Output and Cardiac Size of Giving Digitalis to Patients Suffering from Organic Heart Disease without Signs of Congestive Heart Failure.* By HAROLD J. STEWART and (by invitation) J. E. DETTRICK, N. F. CRANE and W. P. THOMPSON, New York, N. Y.

One of us<sup>1</sup> has already reported before this society certain effects upon the circulation of giving therapeutic amounts of digitalis to subjects whose hearts were normal and also to patients suffering from organic heart disease exhibiting signs and symptoms of congestive heart failure. The following facts emerged from those observations: Exposing normal hearts to the actions of digitalis results in decrease in the size of the heart and decrease in the volume of its output of blood per minute. Giving digitalis to certain patients exhibiting signs and symptoms of congestive heart failure when the rhythm was regular or that of auricular fibrillation, resulted in increase in cardiac output and decrease in cardiac size. Since there is one action of digitalis which is common to these two diverse situations, the normal heart and the enlarged heart, namely, an effect on the size of the heart, that is to say a decrease, we were led to the notion that digitalis decreases the size of the heart, and the cardiac output which results depends upon the initial size of the heart; that is, decrease in output in the normal heart and increase in the dilated one. The size of the heart appeared to be pertinent, since we were unable to establish a correlation between decrease in size of heart and decrease in cardiac output and alteration in venous pressure. Results paralleling these had been found to obtain when the observations were made on dogs.<sup>2</sup> In addition, when digitalis was given to dogs in which the hearts were large, the consequence of artificially induced mitral insufficiency, but in which there were no signs of heart failure, the effect was similar to that observed in normal dogs.<sup>3</sup>

It remained, therefore, to observe the effect upon these same functions of giving digitalis to those patients suffer-

ing from organic heart disease who either had never experienced congestive heart failure or who exhibited no signs of congestive heart failure at the time of the exposure to the action of digitalis. Such observations form the basis of this report. To 11 patients, all suffering from rheumatic heart disease, *digitalis American Heart Association* 1.6 to 1.8 gm. was given within 24 hours. The rhythm of the heart was regular in all subjects. Measurements of cardiac output were made by the acetylene method of Grollman, of arm to tongue circulation time by the intravenous injection of decholin, of venous pressure by the direct method, and of cardiac size from the measurement of the cardiac shadow in 2 meter x-ray photographs. Observations were made before and at 24 hours after as well as at later intervals after giving the drug. All observations were made with the patients in a basal metabolic state. The doses of the drug were given at the same time of the day to all subjects, and the observations were made at identical times.

The results fell into 3 groups. 1. In certain of the patients the effect of giving digitalis was to *decrease* cardiac size and cardiac output, and to *increase* circulation time; venous pressure did not follow any consistent pattern; that is to say, the effect was similar to that observed in giving the drug to normal individuals.

2. In others the effect was to *decrease* cardiac size and to *increase* cardiac output and to *decrease* circulation time; the venous pressure was not significantly altered; that is to say, a result similar to that observed in patients suffering from congestive heart failure.

And finally (3) in certain instances, the cardiac size, cardiac output, and circulation time and venous pressure remained unchanged, although the alterations of the T waves of the electrocardiogram showed evidence of one digitalis effect on cardiac muscle.

It appears therefore that if digitalis alters the size of the heart, change in its output occurs; its volume output decreases if the heart was beforehand not dilated and it has been made a smaller pump; on the other hand its output increases if the dilated heart has by becoming smaller been made a more appropriate size. The balance of factors which come into play in those instances in which the circulatory functions which were measured showed no alteration, is not known. In certain instances when digitalis was given and the cardiac output decreased, the patients became cyanotic and dyspneic. It appears therefore that patients without obvious heart failure, patients in a similar functional classification as far as clinical data could be evaluated, behave in an unpredictable way on taking digitalis.

The cardiac volume was plotted against the gram meters of work of the left ventricle per beat. Four patients fell into the zone of threatened heart failure according to the correlation demonstrated by Starr,<sup>4</sup> Fig. 2, but these

Output per Minute Following the Administration of Digitalis in Dogs in Which the Heart is Enlarged.

<sup>4</sup> Starr, Isaac, Jr., Collins, L. H., Jr., and Wood, F. C., J. Clin. Invest., 1933, 12, 13. Studies of the Basal Work and Output of the Heart in Clinical Conditions.

<sup>1</sup> Stewart, H. J., and Cohn, A. E., J. Clin. Invest., 1932, 11, 917. Studies on the Effect of the Action of Digitalis on the Output of Blood from the Heart. Part 1. The Effect on the Output in Normal Human Hearts. Part 2. The Effect on the Output of Hearts in Heart Failure with Congestion, in Human Beings.

<sup>2</sup> Cohn, A. E., and Stewart, H. J., J. Clin. Invest., 1928, 6, 53. The Relation between Cardiac Size and Cardiac Output per Minute Following the Administration of Digitalis in Normal Dogs.

<sup>3</sup> Cohn, A. E., and Stewart, H. J., J. Clin. Invest., 1928, 6, 79. The Relation between Cardiac Size and Cardiac

four did not fall into a single group with respect to the effect of digitalis but fell into the three groups; so that a distinction could not be made on this basis. One correlation did arise, however: in three of these four patients in whom the cardiac size was altered by digitalis, whether the cardiac output was increased or decreased, they moved up into the zone of normal cardiac function, in which the cardiac work became commensurate with the cardiac size.

**Conclusions:** It appears that alterations of the cardiac output following the giving of digitalis are associated with alterations of cardiac size. When alterations of size failed to occur, the cardiac output remained unchanged.

It is demonstrated *anew* that decrease in cardiac size and decrease in cardiac output was not associated with decrease in venous pressure. That is to say, additional evidence is presented that decrease in cardiac output which follows the giving of digitalis in certain instances is not a consequence of fall in venous pressure but, so far as one can ascertain, is a consequence of decrease in size of the heart by the action of digitalis on it.

*Intermittent Blood Flow in the Capillaries of Human Skin.* By JAMES BORDLEY, III, MAX H. GROW (by invitation) and WILLIAM B. SHERMAN (by invitation), Baltimore, Md.

The skin over the tibia of normal human subjects has been observed microscopically. Room temperature has been controlled and the skin-area under observation has been supported horizontally at heart level. Using a magnification of  $90\times$  to  $150\times$  it has been possible to see blood flow in capillary loops, in venules and, occasionally, in arterioles. The flow of corpuscles in many capillary loops has been found to be intermittent in character. Intermissions may be only momentary but frequently last for periods of several minutes or longer. When flow ceases the capillary usually contains motionless corpuscles and remains visible; only occasionally does it disappear from view. Alternating progression and cessation of corpuscular flow has been observed in single capillaries over periods as long as one hour. When flow is observed simultaneously in two or three neighboring capillaries it is generally found that the periods of intermission do not coincide.

We conclude that the blood flow in the capillaries of normal human skin may show intermittency similar to that described for the capillaries of various organs of lower animals (kidney, muscle, lung, etc.). This conclusion differs from that which Lewis<sup>1</sup> derived from his observations upon the skin of the hand and forearm.

*Chemical Studies in Addison's Disease.* By J. S. L. BROWNE and ELEANOR M. VENNING (introduced by J. C. Meakins), Montreal, Canada.

Studies on sodium, potassium, calcium and nitrogen balances have been made on three cases of Addison's disease over a period of months: and the effect of ad-

ministration of sodium chloride by mouth and of cortical (Eschatin) and pituitary adrenotropic extracts studied. There is some indication that the extracts used influence the potassium balance. The effects of glucose, given orally or intravenously, and of adrenalin and small doses of insulin on the blood sugar were investigated in some cases. Sodium chloride administration sometimes depressed the blood urea below the normal level, the excretion of ingested urea was then delayed. One case died after a mild pharyngitis, he was in negative sodium, potassium and nitrogen balance, and the sodium concentration in the urine decreased in the two weeks between the infection and death. During collapse the sodium, urea and volume of the blood were normal and the blood pressure rose; the blood sugar decreased to 20 mg. per cent. It is felt that a disturbance of carbohydrate metabolism in Addison's disease is not only a secondary terminal event but can act in some cases separately from the mineral-water disturbances to cause collapse.

*The Relief of Human Myxoedema by an Artificial Human Protein.* By WILLIAM T. SALTER, Boston, Mass.

Thyroxin contributes only about one-third of the iodine in thyroglobulin extracted from human glands surgically excised in Boston. As previously reported to this society, however, all of this thyroglobulin iodine is equally potent in relieving myxoedema. Although pure di-iodotyrosine is calorigenically inert, yet, paradoxically, it apparently contributes the major part of the metabolic potency when combined in the thyroglobulin molecule.

When this human thyroid protein is digested with pepsin, it is possible to remove the thyroxin fraction. The remaining solution of di-iodotyrosine peptone is free of protein and can be filtered under pressure through a standard "Cellophane" membrane. Under appropriate chemical conditions, this material may be subjected to peptic synthesis, thus reversing the original digestion process.

The artificial protein resulting from this enzymic synthesis resembles natural thyroglobulin. It fails to dialyze through the standard "Cellophane" membrane. Its iodine content varies from 0.2 per cent to 0.3 per cent. It was found clinically<sup>1</sup> to relieve myxoedema as effectively as thyroglobulin in equivalent-iodine dosage. Thyroglobulin from primary hyperplastic glands and from multiple colloid adenomatous goitres yielded similar artificial proteins.

This type of hormone synthesis promises to explain the high activity of thyroid substance. It also suggests that enzymic synthesis may play an important rôle in the economy of this endocrine.

*A Thyroid Derivative with Greater Calorigenic Activity than Thyroxine.* By W. O. THOMPSON and (by invitation) S. B. NADLER, P. K. THOMPSON and S. G. TAYLOR, III, Chicago, Ill.

By proteolytic digestion of desiccated thyroid, a fraction has been obtained which, when injected subcuta-

<sup>1</sup> T. Lewis, Heart, 1926, 13, 1.

<sup>1</sup> Assays were done in the thyroid clinic of the Massachusetts General Hospital.

neously, possesses greater calorogenic activity per milligram of iodine than thyroxine given by the same route. The following observations are important in the interpretation of this finding: 1. Per milligram of iodine, desiccated thyroid is more active than the amount of thyroxine in it would indicate. 2. Experiments in man have failed to show that l-thyroxine possesses greater activity than d-thyroxine (Salter, Lerman and Means) or that a comparatively simple peptide of thyroxine with a nitrogen:iodine ratio of 0.48:1 possesses any greater activity than racemic thyroxine. 3. The method used to extract thyroxine from the thyroid (heating with alkali) destroys from two-thirds to four-fifths of the gland's activity, whereas it has little effect on thyroxine itself. These data suggest: (1) that the activity of thyroxine may be enhanced by its natural combination, and (2) that the compound which possesses this enhanced activity has a more complex molecule than the thyroxine peptide referred to.

*The Use of Gonadotropic Anterior Pituitary Extract in Women Who Flow without a Premenstrual Endometrium.* By ELMER L. SEVRINGHAUS, and (by invitation) RALPH E. CAMPBELL and FREDERICK L. HISAW, Madison, Wisconsin.

Study of menstrual irregularities has convinced us that there is no typical history associated with any one type of disturbance in the ovarian and uterine cycles. Endometrial biopsies have facilitated definite diagnoses. In some patients there was repeatedly a bleeding from an estrin type of endometrium, or anovulatory menstruation. This might occur with regular or irregular cycles.

Since the obvious deficiency in such patients is the failure to form a functional corpus luteum, we have employed the anterior pituitary extracts described and prepared by Hisaw and Fevold. They found that use of this material subcutaneously stimulated development of Graafian follicles, but that luteinization in the monkey was obtained only after the intravenous administration of the same mixed pituitary extract. We therefore tried this material intravenously in selected patients, at 14 days after the beginning of a flow. Treatment was given once monthly, with doses ranging from 0.5 to 1.0 cc. of the extract such as is now on the market.<sup>1</sup>

We can report that in at least four such patients we have secured indubitable evidence of the formation of a corpus luteum with typical progestational endometrium. In four others this did not follow although there was clinical benefit. These latter cases came to laparotomy shortly after treatment, and it was found that they had large follicular cysts. In two cases these cyst walls showed evidence of beginning luteinization, but not complete corpus luteum formation. The results accord with the findings of Hisaw in monkeys. He was unable to cause luteinization after he had stimulated the ovaries

to the extent of producing follicular cysts of large size. We believe this is the first evidence of the production of corpora lutea in women by the use of anterior pituitary extracts.

*The Pathological Physiology of the Menopause.* By FULLER ALBRIGHT, Boston, Mass.

This study consists of data which enable one to plot the pituitary prolactin A and estrin contents of the urine of patients at the menopause against the number of hot flashes. The data were collected before, during and after treatment with estrin. Certain deductions are made. In the first place the menopause is a "primary ovarian amenorrhoea" as opposed to lack of ovarian function secondary to pituitary hypofunction, because with the under-production of estrin, there is over-production of prolactin A. The next question, the one which this study especially concerns itself with, is which of these hormonal abnormalities, if either, is the cause of the systemic symptoms. Evidence is brought forth that lack of estrin is not the cause of the hot flashes; however, the evidence is convincing that estrin therapy stops the hot flashes; estrin therapy also decreases the prolactin A over-production; the evidence is not altogether conclusive, however, that the hot flashes are directly correlated with the excessive prolactin A production. Certain implications as to treatment are made.

*Respiratory Function in the Respiratory Neuroses.* By RONALD V. CHRISTIE, Montreal, Canada.

In the past 2½ years, thirty-five cases suffering from one or other form of respiratory neurosis have been collected from the patients passing through the Royal Victoria Hospital. The identification and classification of these cases has been facilitated by the taking of spirometric tracings of the tidal air and of various forms of respiratory gymnastics. In some cases it was only by means of such tracings that a final diagnosis of a respiratory neurosis could be made. The two main groups which could be defined by this technic were: (a) the anxiety neuroses, which include cases of effort syndromes and so-called war neurasthenia and (b) the conversion hysterics which in their later stages give rise to "hyperventilation tetany." An analysis of haemo-respiratory exchange in selected cases from each of these two groups is of interest in showing the physical changes which may result from such a functional disturbance.

*Carotid Sinus Syncope and its Bearing on the Mechanisms of Unconscious State and Convulsions.* By EUGENE B. FERRIS, JR. (by invitation), and RICHARD B. CAPPS (by invitation) and SOMA WEISS, Boston, Mass.

Weiss and Baker have demonstrated the relationship existing in man between a hypersensitive carotid sinus reflex and certain types of syncope and convulsions. They have differentiated three types of syncope of carotid sinus reflex origin. Prolonged cardiac asystole with a secondary fall in the arterial pressure, and a primary re-

<sup>1</sup> For the supply of the pituitary material we are indebted to Dr. A. E. Meyer, of Chappel Bros. Laboratories, Rockford, Ill.

flex vasodepressor reaction without cardiac inhibition, respectively, have played a primary rôle in two types. Cerebral anoxemia was a precipitating factor in both of these types.

This study presents the results of an investigation of the third, and hitherto obscure, type of syncope, as manifested in 25 patients observed in spontaneous and in induced attacks. The following observations indicate the specific sensitivity of the sinus: 1. Pressure over the sinus induced attacks in as short a time as 4 seconds. 2. Sudden increase in the intracarotid pressure resulting from the release of a compressed carotid artery below the sinus, induced mild symptoms. 3. Stimulation of the sinus with sodium cyanide resulted in symptoms. 4. Occlusion of the carotid artery below the sinus failed to induce symptoms. 5. Novocainization of the sinus abolished the induced reactions. 6. Surgical denervation of the sensitive sinus abolished both the spontaneous and the induced symptoms in 8 patients for as long as 14 months.

The clinical manifestations cannot be explained on the basis of changes in the systematic hemodynamics during attacks, as no fall in blood pressure nor cardiac slowing occurred. The cerebral circulation during and between attacks was studied with the aid of the Gibbs blood-flow recorder, as well as with blood gas measurements. Observations were also made on the effects of strychnin, digitalis, oxygen, carbon dioxide, acetyl- $\beta$ -methylcholin, caffein, amyl nitrite, ephedrin, adrenalin, pilocarpin and atropin.

The interpretation of the observations in this study is that the manifestations were brought about not by generalized cerebral anoxemia, but by nervous impulses originating in the sinus and acting upon a localized area of the midbrain regulating consciousness. The observations bear pertinently on the reflex origin of unconscious states and on the mechanism of the tonus of autonomic nerve centers.

*The Incidence and Significance of Minute Beta Hemolytic Streptococci.* By PERRIN H. LONG and (by invitation) ELEANOR A. BLISS, Baltimore, Md.

Minute beta hemolytic streptococci have been isolated from the throats of 87 per cent of 66 individuals ill with glomerular nephritis. It is noteworthy that these organisms occurred with much greater frequency in those individuals whose disease had had an acute onset than in those in which the onset of the nephritis was insidious. These minute organisms were isolated from the throats of 50 per cent of individuals suffering from rheumatic (fever) infection. In both glomerular nephritis and rheumatic infection more throat cultures were positive for minute beta hemolytic streptococci than for ordinary beta hemolytic streptococci. The incidence of these minute organisms in the throats of normal human beings and in those of patients ill with diseases other than nephritis or rheumatic infection was less than that of ordinary beta hemolytic streptococci.

When tested by Lancefield's method these minute organisms fall into three antigenic groups which are different from those described for ordinary beta hemolytic

streptococci. They produce a filterable hemolysin which differs from that already described for ordinary beta hemolytic streptococci. The sera of certain normal individuals contained agglutinins in low titer for these organisms. The sera of patients suffering from glomerular nephritis beginning with an acute onset, streptococcal sore throat and rheumatic infection contained agglutinins in a relatively high titer against these minute organisms. In certain instances recovery from these diseases was paralleled with a marked increase in the agglutinin titer against these minute organisms. The sera of normal carriers of minute hemolytic streptococci showed a normal agglutinin content. In view of the well known association of beta hemolytic streptococcal infections of the throat with the onset and progression of acute glomerular nephritis and rheumatic (fever) infection we feel that these findings assume an added importance.

*The Effect of Splanchnic Nerve Resection and Sympathetic Ganglionectomy in a Case of Paroxysmal Hemoglobinuria.* By A. CARLTON ERNSTENE and (by invitation) W. JAMES GARDNER, Cleveland, Ohio.

In a patient with paroxysmal hemoglobinuria, hemoglobinuria regularly followed the application of ice packs from the feet to the level of the anterior superior spine of the ilium or xiphoid process. After spinal anaesthesia, ice packs did not cause hemoglobinuria. Novocaine block of both lumbar sympathetic chains did not prevent the production of hemoglobinuria by ice packs. For more than one month after resection of the left splanchnic nerves and removal of the first lumbar ganglion, ice packs failed to induce hemoglobinuria; and after a similar operation on the right side, ice packs were ineffective for nearly six months. Subsequent complete lumbar ganglionectomy and cervico-dorsal ganglionectomy did not prevent the production of hemoglobinuria by ice packs although packs of somewhat longer duration were necessary after the operations. Increased tolerance to cold is demonstrated further by a great reduction in the frequency of spontaneous attacks. One year ago short exposure to ordinary winter weather invariably induced hemoglobinuria, while during the present winter there have been but three attacks in spite of prolonged periods of exposure.

The Donath and Landsteiner reaction was consistently positive before the first operation but became negative after that operation and has remained so.

The observations indicate that the sympathetic system plays an important rôle in the pathogenesis of paroxysmal hemoglobinuria.

#### READ BEFORE SECTION B

*Metabolic Studies of the Change in Body Electrolyte and Distribution of Body Water Induced Experimentally by Deficit of Extracellular Electrolyte.* By DANIEL C. DARROW and (by invitation) HERMAN YANET, New Haven, Conn.

Dogs were subjected to losses of extracellular electrolyte by a technique previously described. About 100 cc.

per kilogram of body weight of 5 per cent solution of glucose was injected into the peritoneal cavity and after considerable electrolyte had diffused into the solution as much of the peritoneal fluid was removed as was possible with a trocar. The dogs were fed a food containing little Na, K and Cl, and the serum and whole blood were analysed for electrolyte, and the balance of electrolyte determined over a number of days on the excreta.

The data on the blood demonstrate that deficit of extracellular electrolyte (chiefly Na and Cl) produces a considerable decrease in the volume of the plasma and extracellular fluid. The loss of extracellular fluid is explained not by loss of water from the body but rather by shift of extracellular water into the cells. The blood studies show that this disturbance in the distribution of body water persists until the deficit of Na and Cl is replaced. The body apparently does not compensate for decrease in extracellular electrolyte by decrease in intracellular potassium. Such losses of potassium as occur are accounted for by losses of nitrogen. Furthermore, electrolyte concentration remains reduced since the kidneys fail to excrete water in order to adjust electrolyte concentration. The dogs show persistent signs and symptoms of dehydration which is not accounted for by any loss of water but rather by the disturbance in the distribution of body water brought about by the deficit of extracellular electrolyte.

*Magnesium Balances in Health and Disease.* By DOROTHY M. TIBBETTS (by invitation) and JOSEPH C. AUB, Boston, Mass.

The normal partition of magnesium excretion on a constant diet has been established. As with calcium, magnesium is excreted more in the feces than in the urine. The addition of more magnesium in the diet increased excretion by both channels, but predominantly in the fecal excretion. It also obviously increases urinary calcium excretion.

The production of an acid intake by the use of ammonium chloride and also the addition of various phosphates did not usually affect magnesium output in urine or feces. The effect of hyperparathyroid disease and of parathormone has been carefully studied, and it is clearly demonstrated that calcium and magnesium metabolism are not similarly affected. Thus, magnesium excretion remains comparatively constant, while calcium metabolism varies enormously.

It is, therefore, clear that magnesium metabolism is not markedly affected by the agents which influence calcium exchange.

*The State of Calcium in the Blood in Nephritis and Uremia.*<sup>1</sup> By FRANKLIN C. McLEAN and LOUIS LEITER, Chicago, Ill.

When the phosphate concentration of the blood is augmented in normal animals there is, first, formation of a

colloidal calcium-phosphate complex, resulting in a fall in  $\text{Ca}^{++}$  concentration; and, second, removal of the complex from the blood, resulting in a fall in total calcium.<sup>2</sup>

When  $\text{Ca}^{++}$  concentrations as observed by the frog heart method are compared with those calculated from total calcium and total protein, in cases of uremia with hyperphosphatemia, no indication of accumulation in the blood of a calcium-phosphate complex is found. In nearly every case, however, in which the inorganic phosphate concentration of the serum exceeds 3.0 millimols per liter the  $\text{Ca}^{++}$  concentration is depressed below the normal level, associated, in some cases, with tetany. No satisfactory further correlation between the phosphate level and the  $\text{Ca}^{++}$  concentration has been found.

These findings are best explained, qualitatively, by assuming that a colloidal calcium-phosphate complex is formed in the blood, in the presence of hyperphosphatemia, and is removed from the blood as rapidly as formed. The quantitative relationships involved are not as yet clear.

*The Volume of the Extracellular Fluids.* By P. H. LAVIETES, (by invitation) J. BOURDILLON and (by invitation) J. P. PETERS, New Haven, Conn.

The extent of diffusion of sucrose, thiocyanate and inorganic sulfate in man and the time required for its completion have been determined. All three substances can be recovered quantitatively from the urine. Of the three, SCN alone enters red blood cells, and even this probably enters no other cells in the body. In normal subjects, sucrose and inorganic  $\text{SO}_4$  diffuse through approximately 20 per cent of the body weight; SCN, through 22 per cent. This portion probably represents extracellular fluids of the body and changes when those fluids are expanded or contracted by varying the salt intake. One and one-half hours is sufficient for complete diffusion of sucrose; SCN and  $\text{SO}_4$  diffuse more rapidly. In 4 patients with advanced renal failure without edema, extraordinarily high values were attained, probably because extracellular fluid had replaced wasted cellular tissue and fat.

*Studies of Hypoproteinemia and Proteinuria.* By HAROLD A. BULGER, St. Louis, Mo.

Hypoproteinemia in nephritis usually continues and may remain relatively constant even with considerable nitrogen storage over a long time. That protein is not lost into extra-vascular fluids and then rapidly broken down has been indicated by a study of the amino acids of these fluids. The amino acid content is continually about the same as that of serum. An increased protein intake, although attended by an increased nitrogen storage, results in a greater proteinuria and unchanged plasma protein level. Indirect evidence of defective protein formation is presented. This with the variability of proteinuria is pictured as explaining the continued hypoproteinemia. Factors influencing proteinuria are there-

<sup>1</sup> This work has been aided by a grant from the Josiah Macy, Jr., Foundation.

<sup>2</sup> McLean, F. C., and Hinrichs, M. A., Proc. Am. Soc. Biol. Chem., 1935 (in press).



fore important therapeutic considerations. Urea administration, like comparable amounts of protein, may result in considerable nitrogen storage but increased proteinuria. Diuresis of alkaline potassium salts results in increased proteinuria. Sodium bicarbonate may cause an increase in protein excretion. Mercurial diuretics may increase the protein loss. The rise in nitrogen metabolism from insufficient calories was attended by greater proteinuria.

*Elimination of Heat by Evaporation of Water from the Lungs in Heart Failure.* By J. M. STEELE, New York, N. Y.

It has been shown that during heart failure the surface temperatures of certain individuals are low while after recovery they rise (Steele and Cohn). If identical environmental conditions prevail less heat is radiated from the surface before than after recovery. Evidently heat is lost elsewhere. The result of this study suggests that the lungs redress the balance.

The patients in a basal state were examined under relatively constant environmental conditions. They breathed into a Benedict-Roth spirometer supplied with a rotary pump. Water and  $\text{CO}_2$  eliminated by the lungs were taken up by  $\text{CaCl}_2$  and soda lime respectively and weighed. Consumption of oxygen was measured volumetrically. The tests lasted 12 minutes. Duplicate measurements differed by less than 5 per cent. The accuracy of the apparatus was within 1.2 per cent (alcohol check lamp).

In two normal persons and in two with arterial hypertension heat eliminated by vaporization of water from the lungs was found to vary from 9.5 to 12 per cent of the total heat produced by the body. These figures agree well with those of Benedict and Benedict (1926). In three cardiac cases, in which the surface temperatures were shown to be low during heart failure, the percentile values were thrice normal (30 to 35 per cent) and fell to normal (10 to 15 per cent) with recovery. In the fourth case, one of recent mild heart failure, the fall was not so marked (from 16 to 11 per cent), and the surface temperatures were not low.

It is concluded that loss of water from the lungs increases during heart failure to a greater extent than does the general metabolism, acting apparently as a substitute for radiation from the surface of the body.

*Effects of Amino-acids on the Creatine Content of Rabbit Heart Muscle.* By GEORGE DECHERD (by invitation) and GEORGE HERRMANN, Galveston, Texas.

The isolated rabbit heart perfused with oxygenated Ringer-Locke solution will fail in 6 to 10 hours even to artificial stimulation and in spite of the removal of the  $\text{CO}_2$  from the perfusate and maintenance of the pH at the normal alkaline level. The creatine content of the heart muscle dropped when the pH of the perfusion fluid was allowed to fall below 7.3-7.5. Twenty-three normal hearts perfused for one minute to wash out the coronary system contained an average of 154 mgm. per cent (143-168 mgm. per cent) of creatine. Fifteen hearts perfused for one to eight hours beating spontaneously, or stim-

ulated at a rate of 60 per minute to exhaustion, showed 100-151 with an average of 123 mgm. per cent of creatine. Dried weight values were calculated for all hearts and were found to parallel the moist weight values. Glycocoll added to the perfusate in 100 mgm. per cent augmented the action of the hearts in 21 experiments but had no influence on the creatine content, which showed values of 103-159 with an average of 134 mgm. per cent. Glutamic acid was toxic in 100 mgm. per cent in the perfusate but, after 10 mgm. per cent was added to the perfusate, 4 hearts contained 108-134 with an average of 116 mgm. per cent of creatine. Arginine in 10 mgm. per cent was added in a similar series and gave 100-126, average 115 mgm. per cent; aspartic acid in 10 mgm. per cent in the perfusion fluid had little effect with creatine values of 108-148 with an average of 134 mgm. per cent; methyl-guanidine in 10 mgm. per cent was also without definite effect, since levels of 126-150 with an average of 138 mgm. per cent were obtained; while with creatine itself the results were 125-161 with an average of 138 mgm. per cent. d-l-Alanine, however, in 50 or 100 mgm. per cent in the perfusate seemed definitely to raise the creatine content, the results in 20 experiments being 136-205 with an average of 166 mgm. per cent. This amino-acid therefore appears to act as a sparer of creatine in the rabbit heart muscle under the conditions of our experiments. Myocardial infarction, regardless of the perfusate used, caused a very sharp drop in the creatine values, these being 51-99 with an average of 79 mgm. per cent.

*The Effect of Exercise on the Blood Sugar of Patients with Diabetes Mellitus With and Without Insulin.* By ALEXANDER MARBLE and (by invitation) RACHEL M. SMITH, Boston, Mass.

Exercise along with diet and insulin is an accepted part of the present-day treatment of diabetes. Its ability to lower the blood sugar of patients receiving insulin is well recognized. That this beneficial effect may not occur in an individual who is receiving an inadequate amount of insulin is, however, not generally appreciated. Serial determinations of the capillary blood sugar of young diabetic patients were made in the early morning before insulin and breakfast. Suitable control curves were obtained with the patient at rest, with and without insulin, and with the patient exercising with insulin. All tests done without previous insulin were uniform in showing an unmistakable rise in the blood sugar following five or ten minute periods of moderate exercise (running or using a rowing machine). This occurred even though the initial blood sugar was as low as 0.16 per cent as it was in one instance. The conclusion is drawn that in order for exercise to exert its maximum benefit in diabetic patients there must be an adequate supply of insulin either of endogenous or exogenous origin.

*The Effect of Crude Lipoids on Antigenic Function.* By FRANKLIN M. HANGER, New York, N. Y.

Numerous workers have demonstrated that the physical state of an antigen plays an important rôle in the

degree of antibody production. For example, egg albumin adsorbed on alumina or calcium phosphate is more effective as an antigen than an equal amount of aqueous egg solution. This may be due in part to the avidity of the reticulo-endothelial system for particulate matter.

Crude lecithin is non antigenic; it is, however, a most effective emulsifying agent and when injected into the tissues is readily taken up by clasmotocytes and other reticulo-endothelial cells. Experiments were devised to determine if this substance, when mixed with antigens of various types, can modify the immune response.

.45 mg. crystalline egg albumin in solution, mixed with varying amounts of crude Merck lecithin, was injected into guinea pigs. These animals were tested nineteen days later for anaphylactic response to egg albumin. Results show that when the egg solution was mixed with lipoids, an increase in sensitivity resulted. In this case the lecithin presumably acts as an adsorbant. Other animals were injected with similar amounts of albumin precipitated with alum and mixed in like manner with varying amounts of lecithin. The effect of the lipoid in this case was quite opposite. Its presence in suitable proportions inhibited the development of sensitivity when the antigen was in particulate form.

This problem has also been investigated in another manner. Six day chick embryos have been shown by Dochez and Kneeland to be relatively non-antigenic. This observation has been confirmed by injecting guinea pigs with various amounts of whole embryo in saline suspension and shocking the animal nineteen days later with the same material—only slight reactions were noted. When, however, the embryos were extracted with alcohol, which removed most of the lipoids, the remaining material was highly antigenic. When the alcohol soluble portion was restored to the extracted fraction the mixture was relatively non-antigenic.

*The Clinical Significance of Chorea as a Manifestation of Rheumatic Fever: a Study in Prognosis.* By EDWARD F. BLAND (by invitation) and T. DUCKETT JONES, Boston, Mass.

An analysis of the prognostic significance of chorea as a manifestation of rheumatic fever has been made in 1000 rheumatic fever and chorea subjects. The results do not confirm the general impression that Sydenham's chorea is a grave manifestation of rheumatic fever.

Chorea occurred in 482 patients. Of these (over an average period of 8 years) 72 per cent exhibited other rheumatic fever manifestations, while 28 per cent had chorea alone.

The incidence of rheumatic heart disease in the above groups was:

- A. Rheumatic fever (without chorea) 86 per cent.
- B. Total chorea group 54 per cent.

Further analysis of this group (B):

1. Chorea with other manifestations of rheumatic fever, 73 per cent
2. Chorea without other manifestations of rheumatic fever, 3 per cent.

The severe manifestations of rheumatic fever (especially precordial pain, pericarditis, and congestive failure) occur twice as frequently in patients having rheumatic fever as in those having chorea in addition to rheumatic fever.

Over an 8 year period death occurred in only 0.7 per cent of the cases with chorea alone; in 14 per cent in the rheumatic fever and chorea group; and in 32 per cent of the straight rheumatic fever group.

Chorea is hence considered a mild manifestation of rheumatic fever and one not especially conducive to the development of rheumatic heart disease.

*Studies in Streptococcal Immunity:—Erysipelas.* By WESLEY W. SPINK (by invitation) and CHESTER S. KEEFER, Boston, Mass.

As a part of an investigation of the response of the body to  $\beta$ -hemolytic streptococcal infection, patients with erysipelas have been studied. The investigation has been divided into two parts. 1. A survey of the various epidemiological, clinical features and course of the disease as it occurs in the Boston City Hospital. This included a review of 1400 cases with special reference to age and seasonal incidence, site of lesion, complications, mortality and factors influencing the outcome of the disease. 2. A small group of patients with erysipelas were studied to determine the time of the appearance of antibodies as measured by: (1) whole blood killing power; (2) anti-streptolysin; (3) anti-fibrinolysin; (4) complement; (5) agglutination reactions; (6) skin reactions.

It was found that the general mortality in the group of patients with erysipelas was 16.2 per cent and this was influenced by age and debilitating diseases. The death rate was highest in the 1st, 5th, 6th and 7th decades, whereas the morbidity was highest in the 3rd and 4th decades. The highest death rate occurred under one year of age, and the lowest in the second and third decades. With this information at hand, an attempt was made to determine the presence of antibodies in the circulating blood during the course of the disease. It was found that the whole blood killing power for the patient's own organism increased during and after the disease had subsided; there was also an increase in the anti-streptolysin titre of the blood serum and an increased resistance of fibrinolysis. There were no consistent fluctuations in the titre of the complement, but it was more common to observe a diminishing titre in the blood serum during the course of the disease and a return to the previous level following recovery. Agglutinins to the patient's own organism were not detected during or following recovery. The skin reactions to the streptococcal nucleoprotein showed a positive reaction in only 66 per cent of the cases.

From the observations so far it is possible to state that the recovery from erysipelas is associated with, and followed by, the development of antibodies to the infecting organism. There is, in addition, evidence that the presence of circulating antibodies will not prevent



the development of recurrent attacks of erysipelas nor prevent the invasion of the blood stream from the local lesion. Further studies are being carried out to gain more information regarding the recovery process and the immune mechanism involved.

*The Nature of the Secondary or "T" Process of the Electrogram of Cardiac Muscle.* By A. GARRARD MACLEOD, New York, N. Y.

The normal form of the secondary or "T" process of the electrogram and the effect of local cooling on this process have been studied in the frog's auricle. The results obtained can be explained by a mathematical analysis similar in principle to that used by Wilson, Macleod and Barker in explaining the primary deflection of the electrogram of the dog's auricle. However, a simple graphical scheme has been devised which has been found to be much simpler and more convenient. From this analysis it appears that the velocity of spread of both the primary and secondary processes is the same, but that the potential differences which give rise to the secondary or "T" deflection are of opposite sign, smaller magnitude and much longer duration than those which give rise to the primary deflection. While it was found convenient to use the frog's auricle for most of the experiments upon which this work is based, it can be shown that the process is essentially the same in other varieties of cardiac muscle. It is believed, therefore, that it will be possible to substitute for the purely empirical interpretation of the "T" deflection one which explains such changes as take place in it in terms of the velocity with which the process spreads and the magnitude and duration of the potential differences which develop in the muscle units.

*Observations on the Components of Split First Heart Sounds.* By CHARLES C. WOLFERTH and ALEXANDER MARGOLIES (by invitation), Philadelphia, Pa.

We have recently presented evidence to indicate that when the first heart sound is split, there is asynchronism in ejection from the two ventricles. This finding may be regarded as supporting but not actually proving the postulate that one component of sound originates in each ventricle. In view of the fact that, as had been previously shown, the loudness of the first heart sound is greatly influenced by the As-Vs time relation, it seemed possible to test the validity of the postulate in cases exhibiting both split first sounds and varying As-Vs intervals. Long strips of simultaneously recorded sound tracing and electrocardiogram were made in two cases with complete heart block, and split first sounds. In both of these cases it was noted that during a certain range of As-Vs time intervals the two sound components varied independently of each other. The variation in each component depended on its own time relation to auricular contraction.

This behavior of the two sound components is in harmony with the postulate that one component is produced in each ventricle. As a matter of fact, the independent

variations in the two components appear to exclude the possibility that both originate in the same ventricle.

Fragmentary observations in other cases with split first heart sounds and varying As-Vs intervals showed behavior similar to that of the two completely studied cases. Furthermore, in a case with a markedly prolonged, but not split, first sound the largest recorded vibrations occupied the first or second half of the first sound depending on the As-Vs time relation.

These observations support the view that the important duality of single as well as split first sounds consists of components contributed by the two ventricles.

*The Quantitative Relationship Between the Amount of the "Intrinsic Factor of Castle" and the Maturation of Red Blood Cells in Patients with Pernicious Anemia.* By S. M. GOLDHAMER (by invitation), RAPHAEL ISAACS and CYRUS C. STURGIS, Ann Arbor, Mich.

If the intrinsic factor of Castle is absent from the gastric juice of patients with pernicious anemia, there are two questions to be answered: (1) Why do any red blood cells mature in these patients? and (2) Why do these patients, in relapse, have different red blood cell levels? The secretion of gastric juice, with or without histamin injection, in 176 collections from 26 patients with pernicious anemia in relapse, was studied. The average secretion per hour in these patients was 20 cc. as compared with 150 cc. per hour in normal individuals under the same conditions. Gastric secretion collected from untreated patients with pernicious anemia was incubated with ground beef steak and then fed daily to two patients with pernicious anemia. A third pernicious anemia patient was given intramuscular injections of his own gastric juice. Data on the blood changes which followed are given. The response suggests that patients with pernicious anemia do secrete the precursor of the hematopoietic stimulant, but it is deficient in quantity. Further analysis reveals a direct relationship between the gastric juice volume and the red blood cell level in relapse, that is, the less the amount of gastric secretion, the lower the red blood cell count.

*Hematopoietic Material from Liver Active in Pernicious Anemia.* By H. D. DAKIN (by invitation) and R. WEST, New York, N. Y.

The preparation from commercial liver extract of products clinically active in causing remission in pernicious anemia is described. The method is based on the removal of much relatively inactive material by precipitation with alcoholic calcium acetate, followed by precipitation of the active material with Reinecke acid. The decomposition of the Reineckate requires special methods which are described. Subsequent purification is effected by "salting out" the active material with ammonium sulfate and later by the use of either magnesium sulfate or of flavianic acid. Thirty mgm. of the product caused a perceptible reticulocyte response in suitable pernicious anemia patients while 80 mgm. have given a maximal response.

Under suitable conditions substantially the whole of the active material is precipitable by ammonium sulfate since none could be recovered from the filtrate. Precipitation in the magnesium sulfate is quantitatively less complete. The yield of purified product approximates 1 per cent of the dry liver extract.

The clinical activity of the products is readily abolished by exposure to cold  $\frac{1}{2}N$  sodium hydroxide and by boiling for 1 hour with  $\frac{1}{2}N$  sulfuric acid and also by salts of heavy metals. Exposure to alkali involves extensive racemization.

On hydrolysis of the active material the following products were obtained. (a) An aminohexose similar to glucosamine but not definitely identified as such. It gave phenylglucosazone on treatment with phenylhydrazine and pyrrol derivatives on condensation with acetylacetone and ammonia on treatment with alkali. Chondrosamine was absent. (b) The following amino acids were present in all preparations so far obtained: lysine, arginine, glycine, leucine, hydroxy, proline, aspartic acid. Crude preparations contained in addition histidine, glutamic acid, glycine and possibly traces of phenylalanine. No other carbohydrate or amino acid groupings were detected. The product, unlike glucosamine, gives a positive Molisch reaction, but we find that glucosamine peptides behave similarly. Pyrimidine or purine bases were absent. No claim to strict chemical individuality is advanced since the separation of aminohexose polypeptides must of necessity be extremely difficult.

*The Multiple Nature of the Pernicious Anemia Principle in Liver.* By CYRUS H. FISKE (by invitation) and Y. SUBBAROW (by invitation), and BERNARD M. JACOBSON, Boston, Mass.

A preliminary report of the response of the guinea pig's reticulocytes to materials which are effective in pernicious anemia was presented before this society last year. Since that time the indirect evidence that the guinea pig's response is related to substances which are concerned with human hematopoiesis has been strengthened by the fact that the liver of a second patient, dying in a relapse of pernicious anemia, has proven quantitatively almost completely inert in the guinea pig.

But in addition to the indirect evidence for the validity of the guinea pig phenomenon, more direct evidence has been obtained. During the past few years work has been in progress on the isolation from the liver of the therapeutically active substances. The successive liver fractions derived by chemical procedures have been tested on both guinea pigs and on patients. Both methods of testing yielded corresponding results, as long as a high degree of purification was not attained. But on further purification, fractions were obtained which were positive in the guinea pig, but which were devoid of therapeutic activity. To date there have been separated from liver at least three different fractions, of which two have been brought to a crystalline state. The separate guinea pig activities of each of the three fractions total about 95 per cent of the guinea pig activity of the crude liver

extract used as a starting point in the separation. Each of the three fractions exhibits a quantitatively different degree of guinea pig activity, but each fraction, when separately administered by the intramuscular route to the patient suffering from pernicious anemia, is therapeutically completely inert. On the other hand, a mixture of the fractions is therapeutically highly active.

The above facts furnish, therefore, direct evidence for the biological validity of the guinea pig phenomenon and indicate that the therapeutic action of liver in pernicious anemia depends upon the combined effect of at least two substances.

*The Relation of Fluctuations in the Number of Reticulocytes in the Guinea Pig to the Injection of Liver Extract.* By LOUIS S. GOODMAN (by invitation), ARTHUR J. GEIGER (by invitation) and THEODORE G. KLUMPP, New Haven, Conn.

In the last proceedings of this Society, Jacobson described a "pernicious anemia-like state" in the guinea pig in which a specific reticulocytosis was induced in certain of the animals by the injection of potent liver extracts.

In attempting to utilize this phenomenon for the assay of liver extracts we found that when suitable animals with low reticulocyte counts were injected with potent extracts, a reticulocyte increase followed in 40 per cent of the experiments. However, the same response occurred in reactive animals after the injection of normal saline. Moreover spontaneous abrupt increases in the number of reticulocytes were so common that eventually the tenuous distinction between reactive and non-reactive animals was usually destroyed. It seemed highly probable that all the reactions were either non-specific or spontaneous in nature. A total of 141 tests on 69 guinea pigs was made.

On theoretical grounds there is no physiological basis for the development of a reticulocytosis after liver in guinea pigs with normal blood counts, any more than that such a phenomenon should occur in normal human beings or in pernicious anemia patients with normal blood counts. Moreover the spontaneous fluctuations in the number of reticulocytes in the normal guinea pig are of such a character that the use of this phenomenon for the bioassay of liver extracts is extremely hazardous.

*Physiological "Macrocytic Anemia" of the Fetus.* By M. M. WINTROBE and (by invitation) H. B. SHUMACKER, JR., Baltimore, Md.

It is well known that the red corpuscles of the newborn are larger than those of the normal adult. Little study has been made, however, of the size and number of the corpuscles in the fetus. Examination of the blood of feti of man, the pig, rabbit, rat, cat and dog, has revealed that macrocytosis is more marked and the red cell count is lower, the less mature the fetus. In comparison with the blood of the adult, there is found in the fetus what may be called "macrocytic anemia." As the fetus develops, the erythrocyte count rises and the size

of the cells diminishes. The proportion of nucleated red corpuscles decreases rapidly, but the percentage of reticulocytes falls more slowly. In many respects, the blood of the mammalian fetus resembles that of cases of pernicious anemia which are being subjected to an effective, continuous and extremely potent stimulus. It seems plausible that this stimulation is afforded by a substance similar to, or identical with, the antianemic principle of Castle. Since, presumably, there is no gastric digestion in the fetus and no combination of "intrinsic" and "extrinsic" factors occurs, it is probable that the effective material passes from the stores of the mother to the fetus through the placental circulation. The biologic relationship of fetal hematopoiesis and that observed in pernicious anemia during liver therapy is being investigated.

*The Effect of Irradiation on Platelet Production in Patients with Essential Thrombocytopenic Purpura Hemorrhagica.* By STACY R. METTIER and (by invitation) ROBERT S. STONE, with the technical assistance of KATHERINE PURVIANCE, San Francisco, Cal.

Six patients with essential thrombocytopenic purpura comprised a series for this study. Prior to treatment the platelets were about 10,000 per cu. mm. in one patient and varied between this figure and 40,000 in the others. All patients received x-ray therapy over the spleen of 200 R units, and in one patient who had a recurrence of symptoms 300 R units were later used. The patients received a total of from 1260 to 3300 R units. Four of these patients showed an increase in platelet production beginning within 24 hours and going up as high as from 250,000 to 400,000 per cu. mm. in 4 to 5 days. In one patient with chronic thrombocytopenia of two years duration no effect was shown following liver extract therapy, multiple transfusions, large doses of iron and ammonium citrate daily, high calcium diet, spleen marrow or Lextron; but a definite rise in platelets occurred following x-ray treatment. In one patient a high protein diet failed to increase platelet production, but there was a rise after x-ray treatment. In two patients no increase of platelets was produced by x-ray, ascorbic acid, anti-vennin, multiple transfusions or splenectomy, and the patients died as the result of hemorrhage.

*The Haematology in Multiple Sclerosis.* By PHILIP SOLOMON (by invitation), MARY E. DAILEY (by invitation) and TRACY J. PUTNAM, with the technical assistance of RUTH G. COUGHLAN, Boston, Mass.

Various features of the blood have been studied in patients with multiple sclerosis. It has been shown that the blood clotting time decreases following such stimuli as subcutaneous adrenalin or intravenous typhoid vaccine, and that the decrease is more marked and more prolonged in patients with multiple sclerosis than in control patients. Further studies have been made in the endeavor to throw light on this increased lability of the blood clotting time. The following chemical determinations were made and found to be normal: sodium, chloride, calcium, phos-

phorus, fluorin, nonprotein nitrogen, total protein, albumin, globulin, albumin-globulin ratio, total solids and sugar. Analyses of the arterial and venous blood oxygen and carbon dioxide content were also within normal limits. The blood fibrin content was found to be high in a large proportion of the patients with multiple sclerosis. The blood platelets were frequently found to be increased in these patients. Prothrombin determinations were within normal limits. Brickner's experiments on blood lipase were repeated, and the results found to be inconclusive.

The abnormal gold sol curve in the spinal fluid of multiple sclerosis was shown to be due to the globulin contained therein. Since the blood globulin is not increased in multiple sclerosis, a qualitative study of the globulin is in progress. It has already been shown that solutions of blood globulin from patients with multiple sclerosis produce "paretic" gold sol curves, as compared to moderate "mid-zone" curves obtained from solutions of globulin from control patients.

These findings lend support to the hypothesis that multiple sclerosis is a disease due to multiple small venous thrombi in the central nervous system.

*Nutritional Deficiency and Water Retention in the Toxemias of Pregnancy.* By MAURICE B. STRAUSS, Boston, Mass.

Water retention is generally recognized as a common concomitant of true toxemia of pregnancy and probably occurs to a certain extent in normal pregnancy. Three of the many factors involved in water retention in the non-pregnant individual, the oncotic pressure of the plasma proteins, the venous pressure, and the nutritional state, have been investigated in pregnancy.

The average oncotic pressure of the plasma proteins in 10 patients with eclampsia was 175 mm. of water; in 20 patients with non-convulsive toxemia of pregnancy, 215 mm.; in 15 normal pregnant women who had partaken of low protein diets 232 mm.; and in 20 normal pregnant women who had eaten 60 to 100 grams of protein daily, 258 mm.

The average venous pressure of 20 normal pregnant women was 10 cm. of water and of 20 women with non-convulsive toxemia of pregnancy 13.3 cm. of water, a statistically insignificant difference. The average venous pressure of both groups, however, was at least double that of normal non-pregnant subjects.

Dictary histories of the 20 women with non-convulsive toxemia revealed low protein intakes, frequently over a period of years. Gastro-intestinal disturbances were common. Fifteen of these women were given a diet consisting of 260 gms. of protein, 150 gms. of carbohydrate and 70 grams of fat. This included 300 grams of raw liver pulp daily. Ten of the women received daily intramuscular injections of vitamin B<sub>1</sub> concentrate and of liver extract of the type commonly employed in the treatment of pernicious anemia. This material is believed to contain fractions of the vitamin B complex not present in the B<sub>1</sub> concentrate. These injections were given be-

cause of a possible relationship between a deficiency of vitamin B and water retention such as may occur in beri-beri. Edema disappeared and weight was lost uniformly by these 15 patients. In no case did the arterial blood pressure rise, nor was there any fetal mortality after the institution of this treatment. Headache and visual disturbances abated. Albuminuria did not increase. The non-protein nitrogen of the blood did not rise above 35 mgm. per 100 cc. The oncotic pressure of the plasma proteins rose an average of 7 per cent.

Five of the 20 women with non-convulsive toxemia of pregnancy were given a diet containing 20 grams of protein, 400 grams of carbohydrate and 65 grams of fat. Two patients improved, the arterial blood pressure falling to within normal limits, one patient's condition was unchanged, and two showed increasing albuminuria and a further elevation in the arterial blood pressure. Weight changes were not significant. The oncotic pressure of the plasma proteins fell an average of 9 per cent.

These observations suggest that water retention occurs in certain cases of toxemia of pregnancy as a result, among other factors, of a decreased oncotic pressure of the plasma proteins, and that this may probably be relieved or prevented in many instances by the administration of a diet rich in protein and containing all other nutritional factors.

*Studies on the Urinary Proteins Found in Cases of So-called Orthostatic Albuminuria.* By LAY MARTIN, Baltimore, Md.

In the urine of patients diagnosed as having orthostatic albuminuria and in that of one having albuminuria of years duration, it has been found that the protein present is not albumin. It falls within the class of globulins or gluco-proteins, since a carbohydrate radical is constantly in association with it. It has been isolated in a sufficiently pure form for the determination of components; the iso-electric point has been determined, and it has been crystallized. Qualitative studies have been carried out on the so-called acetic acid body often found in the urine of these cases. This also has protein characteristics and is intimately associated with the carbohydrate radical. Full case records of all patients are on hand.

*Experimental Agranulocytosis.* By W. B. CHEW (by invitation), JOHN S. LAWRENCE, and D. J. STEPHENS (by invitation), Rochester, N. Y.

A leucotoxic serum was produced by immunizing rabbits intravenously with a suspension of guinea pig leucocytes containing seventy-five to ninety per cent amphophiles. Hemolysins were absorbed from the anti-serum with guinea pig red blood cells.

Four guinea pigs were given intracardial injections of this anti-serum. The amphophiles disappeared from the blood within five minutes.

Fifty guinea pigs were given a single intraperitoneal injection of this anti-serum. The amphophiles disappeared within one to seven hours and began to reaccumulate in thirty to forty-eight hours. Their reappearance

was not preceded by the appearance of myelocytes or stab forms.

Four guinea pigs received repeated intraperitoneal injections at various intervals in doses from 0.5 cc. to 2.0 cc. Observations upon these animals demonstrated that successive injections of the same amount of anti-serum had less and less effect. When the dose of anti-serum was increased the original effect was obtained. This tolerance could not be removed by administering a desensitizing dose of normal rabbit serum.

Two animals received daily intraperitoneal injections in gradually increasing amounts. This prolonged the absence of amphophiles to four days, after which they appeared in increasing numbers in spite of continued injections.

In all animals the number of lymphocytes in the blood was somewhat decreased during the period of maximum effect of the anti-serum. The monocytes, eosinophiles, and basophiles behaved as they do after injections of normal rabbit serum. The number of red blood cells and platelets and the body weight were not significantly affected.

The amphophiles which had disappeared from the blood could not be found in the tissues of animals sacrificed immediately after their disappearance.

Differential cell counts on the bone marrow of injected animals disclosed almost complete absence of adult amphophiles and a decreased percentage of myelocytes at the end of twenty-four hours. Observations on the bone marrow of repeatedly injected animals disclosed a marked myeloid hyperplasia with a percentage of adult amphophiles which corresponded to the effectiveness with which anti-serum was controlling the number of amphophiles in the peripheral blood.

Microscopic examination of the tissues of injected animals revealed lymphoid and endothelial hyperplasia in the spleen and lymph nodes. There was no definite morphological evidence of damage to other body cells.

*The Cellular Constituents of Normal Human Synovial Fluid with a Consideration of Influencing Factors.* By CHARLES F. WARREN (by invitation), GRANVILLE A. BENNETT (by invitation) and WALTER BAUER, Boston, Mass.

Cytologic examinations have been made on 150 synovial fluids obtained from human knee joints immediately after death from individuals who had had no joint symptoms. Wide variations in the number of nucleated cells and in the percentage of individual cell types were observed. This and additional data are presented in an attempt to establish what variations in the total number of nucleated cells and individual cell type percentages can occur and still be within the limits of so-called normal.

Thus it has been shown:

1. That postmortem intra-articular cellular migration may take place but is not associated with significant variations in cell percentages or any increase in polymorphonuclear leucocytes.

2. That an increase in the number of circulating leuco-

cytes or a marked polymorphonuclear leucocytosis can occur without any reflection of such variations in the synovial fluid cytology.

3. That the increased number of nucleated cells and percentage of phagocytic cells observed in certain instances is best explained by the increased amount of debris resulting from the cartilage defects present.

4. That an increase in the total amount of synovial fluid, as occurs in oedematous patients, results in a reduction of the total number of nucleated cells per cubic millimeter.

5. That high total cell counts and high polymorphonuclear leucocyte percentages are observed in the synovial fluids obtained from patients dying with any type of severe infection.

Thus, by selecting the synovial fluids obtained from patients who had neither oedema nor infection, one is able to construct a table showing the minimal, maximal and average number of nucleated cells as well as individual cell percentages contained in normal synovial fluid. The variations which still occur are best explained as being due to the degree of wear and tear, minor traumata or irritants, to any one of which all joints must be subjected from time to time.

Knowing the cytological variations which can occur in normal synovial fluid and synovial fluid obtained from patients with severe infections, we shall be better able to evaluate the cytological variations we have observed in joint effusions in the various arthritides. Whether this information will allow for a better interpretation of the joint aches and pains so often complained of in individuals with acute infections will depend upon whether or not studies now in progress will show that synovial membrane changes are regularly associated with variations in the synovial fluid cytology.

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A review of available data on the respiratory quotient, arteriovenous blood sugar difference and behavior of inorganic phosphates of the blood in hyperthyroidism indicates increased utilization of sugar by the tissues. This speaks against pancreatic insufficiency as the cause of reduced dextrose tolerance in hyperthyroidism, because diabetes mellitus is characterized by reduced utilization of sugar.

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Thyroidectomy corrected the abnormalities observed

after ingestion of galactose. Psychic factors were ruled out by control experiments with saccharin.

*Conclusions:* Our findings point to the liver as the chief seat of the disturbance of carbohydrate metabolism in hyperthyroidism. This is confirmed by liver function studies and pathological data. Dextrose therapy, especially as a preoperative measure, is indicated on this basis and is supported by favorable clinical evidence.

*Group Examinations for Pulmonary Tuberculosis.* By ROBERT G. BLOCH and (by invitation) BYRON F. FRANCIS and GRACE HILLER, Chicago, Ill.

The absence of symptoms does not indicate the absence of pulmonary disease; physical examination is not adequate in ruling it out. The gradual recognition of these facts has led to roentgenological group examinations in increasing numbers. The superiority of such examinations as against group testing methods with tuberculin is discussed.

Four thousand adults, students and employees, were examined by fluoroscopy or chest films at the University of Chicago. The definite classification of findings as to the activity of diagnosed lesions demands a period of at least six months. A detailed classification comprising the medical aspects of the management of patients has been worked out. The percentage of active tuberculosis in our group was 1.2 per cent. Observations were made on the occurrence of healed (calcified) pulmonary tuberculous lesions (Ghon's tubercles). The incidence of such lesions was found to be considerably less in the younger individuals than in the older ones. This observation, which should be verified in a larger group tends to throw a new light on the significance of calcifications as to infection and reinfection with tuberculosis. The possibility that many of the calcified small lesions are due to adult reinfection seems great.

The group examinations were extended to those who had been in close contact with the individuals found to have active tuberculosis. This procedure was initiated on the basis of our experience that the great majority of cases of adult tuberculosis are due to a massive, exogenous superinfection, rather than to a revival of lesions acquired during childhood.

Roentgenological group examinations of the general population, chiefly of industrial and other special groups, are recommended.

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A constant abnormality found in all cases of essential hypertension has been an excessive response of the blood pressure to stimulation. A standard test, using a simple type of stimulus (ice water at 5° C.), has been evolved for measuring this response. This test, when applied to a large number of normal subjects, those suffering from various types of disease, and those having essential hypertension, shows that there are two types of reactors:

hypo-reactors, or "normal reactors," and hyper-reactors. Members of families of two, three and four generations have been tested by this method, and the familial or constitutional nature of this characteristic over-response is demonstrated in a remarkable degree. Likewise, the constitutional nature of the hypo- or "normal" reaction shows similar familial characteristics.

*A Study of the Mechanism of Circulatory Insufficiency in Sclerodactylia.* By MYRON PRINZMETAL (introduced by W. B. Castle), Boston, Mass.

Raynaud's disease associated with sclerodactylia is more severe clinically, presents a more difficult therapeutic problem and has different experimental vascular reactions than Raynaud's disease without sclerodactylia. We propose that the tight skin in sclerodactylia has a constricting effect on the circulation of the fingers and thereby is an important factor in explaining these differences between Raynaud's disease with normal skin and Raynaud's disease with sclerodactylia.

In sclerodactylia the areas of greatest circulatory deficiency, as determined by skin temperature, occur where the pressure from the skin is greatest. If the skin is relaxed, a definite rise in temperature takes place which disappears when the skin is taut. In 3 cases in which unilateral sympathectomy was performed, no substantial increase in skin temperature occurred, since the tight skin prevented the denervated vessels from opening.

If a tight binding (finger of a small rubber glove) of about the same intensity as the tight skin in sclerodactylia is placed on a normal digit, the abnormal vascular reactions obtained by Lewis may be duplicated. We found by this method that the skin assumes and follows room temperature, does not have the vasodilator reaction upon exposure to cold and has smaller plethysmographic oscillations. Vasodilator impulses induced by the Landis test cause slight or no increase in skin temperature.

In sclerodactylia or in an experimentally bound normal finger, intermittent suction causes an increase in skin temperature, even though sympathectomy has failed to increase the flow. The mechanism of improvement is probably due to relaxation of the tight skin.

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In our experiments the parathyroid deficiency at the time of transplantation was varied from twenty-five to one hundred per cent. After varying intervals the grafts were excised and examined microscopically. Although there were a certain number of failures in each group, we found that the graft survived and in many instances retained its original size and structure for periods up to three months in experiments where the parathyroid deficiency was only twenty-five or fifty per cent as well as in experiments where it was seventy-five or one hundred per cent. Moreover, we found that two separate



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autografts placed at the same time in animals with a parathyroid deficiency of only fifty per cent would both survive for periods up to two months.

Our observations lead us to believe that a pre-existing deficiency or a physiological need of the organism for the tissue in question is not a requirement for the immediate survival of parathyroid autografts. We have not concerned ourselves in this study with the problem of the factors involved in the ultimate survival of such transplants.

*The Aetiology of Agranulocytosis with Particular Reference to the Factor of Drugs.* By WILLIAM DAME-SHEK, Boston, Mass.

The importance of the factor of drugs in the development of agranulocytosis was studied (1) by examination of clinical records in typical cases, (2) by administration of certain drugs such as amidopyrine to recovered patients, and (3) by the administration of amidopyrine and "allonal" to "normal" subjects. Clinical and hematological effects were noted and the question of allergic sensitivity was investigated.

The following facts were established: (1) that various drugs were definitely linked with the development of agranulocytosis; (2) that certain patients showed extreme hypersensitivity (not allergy) to amidopyrine with the reproduction of granulocytopenia; (3) that most (63 per cent) of "normal" subjects developed definite leukopenia and granulocytopenia after administration of amidopyrine or "allonal." The hourly leukocyte counts were greatly disturbed. Upon discontinuance of the drug in these cases striking leukocytosis ("release phenomenon") frequently occurred.

The following conclusions are at present justified: (1) although multiple aetiological factors are undoubtedly operative in many cases of agranulocytosis, the most important one appears to be that of drugs; (2) the exact mechanism of this action is unknown, although an unusual hypersensitivity probably not allergic is apparent; (3) this hypersensitivity appears to affect the normal maturation and release of cells in the bone-marrow with resultant granulocytopenia.

*The Control of the Hypothyroid State After Total Thyroidectomy in the Treatment of Chronic Heart Disease.* By DAVID DAVIS and HERRMAN L. BLUMGART, Boston, Mass.

Approximately seventy-five patients in this clinic on whom total thyroidectomy has been performed for the treatment of heart disease have been kept free from unpleasant symptoms of myxedema at basal metabolic rates approximating minus 30 per cent by the administration of small doses of thyroid. In the majority of cases thyroid medication was required by the end of the third postoperative month, some weeks after the basal metabolic rate had reached its lowest level.

To accomplish the two-fold purpose of maintaining a low basal metabolic rate, with its coincident low basal cardiac work and at the same time not allowing the

patient to suffer from myxedema symptoms (puffiness of face, excessive fatigue, drowsiness, emotional instability, etc.), an appropriate thyroid dosage varying from  $\frac{1}{20}$  to 1 grain daily, usually  $\frac{1}{8}$  to  $\frac{1}{4}$ , was worked out in each case by trial and error. Patients were seen at frequent intervals and also instructed to return to the clinic if any unpleasant symptoms developed. Nervous irritableness, unaccompanied by other untoward symptoms, occurred in a few patients and required immediate thyroid therapy.

Symptoms frequently became less marked during the postoperative course, so that thyroid dosage could be subsequently decreased and in some instances omitted. In practically every patient the untoward symptoms were controlled by properly regulated dosage, the metabolic rate being maintained at approximately minus 30 per cent. The small doses of thyroid necessary did not greatly increase the work of the heart and, therefore, generally did not interfere with the clinical improvement following total thyroidectomy.

*Observations on Coronary Blood Flow in Aortic Stenosis and Aortic Regurgitation.* By JAY C. DAVIS, Minneapolis, Minn.

A heart-lung preparation was used for the study of coronary blood flow. Aortic stenosis and aortic regurgitation were made in such a manner that the valve was not damaged. The blood flow through the cannulated circumflex coronary artery was studied with perfusion from the aorta at pulsatile pressures and from a perfusion bottle at a pressure below, equal to, and above aortic pressures. The coronary flow per minute was measured by means of a stromuhr. With this method the coronary flow per minute in the same heart with a normal aortic valve, with aortic stenosis, with aortic regurgitation and again with a normal valve was measured.

It was found that a diminution in coronary flow occurs in both aortic stenosis and aortic regurgitation independent of the drop in diastolic pressure. This was evident for the decrease in coronary flow occurred in the valve lesions when the circumflex artery was perfused from the pressure bottle set at such a height that the perfusion pressure was 10 to 20 mm. mercury higher than the systolic pressure in the aorta. Furthermore, similar results were obtained using the whole animal.

The explanation of these results requires additional study. To determine the effect of a single cardiac cycle on the coronary flow in aortic valve lesions, experiments now are being conducted by means of a hot wire anemometer.

*The Influence of Auricular Fibrillation on the Course of Rheumatic Heart Disease.* By ARTHUR C. DEGRAFF and (by invitation) CLAIRE LINGG, New York, N. Y.

This paper is the third in the series describing the events in the course of rheumatic heart disease. The records of 644 patients followed to death are used. The first of two studies now in press describes the factors pertaining to age at initial infection, duration of life

and cause of death. The second study describes the influence of valvular lesions on the course of rheumatic heart disease. In this paper the influence of auricular fibrillation on the course of rheumatic heart disease is discussed.

Of the 644 cases, 276 developed auricular fibrillation before death. The others, as far as we could determine, died while still having a sinus rhythm. It was possible, therefore, to compare the length of life in these two groups. The following facts were elicited:

No significant difference was noted in relation to sex. The highest incidence was found among cases with mitral stenosis.

The longer the duration of rheumatic heart disease, the greater the incidence of auricular fibrillation.

In a number of ways it is demonstrated that auricular fibrillation is usually a late manifestation in heart disease and that it is only observed in relatively long standing cases.

Once auricular fibrillation appears, however, the duration of life is rarely longer, on the average, than  $2\frac{1}{2}$  years from the onset of auricular fibrillation.

*The Clinical Standardization of Digitalis.* By F. R. DIEGAIDE and (by invitation) C. L. TUNG and C. W. BIEN, Peiping, China.

In an attempt to achieve quantitative results in the standardization of digitalis in man it was decided to rely chiefly, and in the end entirely, on objective observations capable of measurement. Clinical congestive failure introduces many uncontrollable variables; it was, therefore, eliminated from consideration. The samples of digitalis to be tested were given at suitable intervals to the same subjects under conditions as closely similar as possible. To test the possibility of separating different potencies of the drug the same doses of three strengths of a standard preparation (related as 75:100:125) were given. (So far as is known the capacity of methods for clinical standardization has not been so tested before.) The samples were supplied as "unknowns," A, B, and C, by the Department of Pharmacology, and only after our conclusions were reached were further data available. The observations included symptoms, blood pressure and electrocardiographic measurements. Among them only the length of the "Q-T" interval in relation to the length of the cardiac cycle seemed to offer the possibility of quantitative separation of the samples of digitalis. Through the changes in this factor it was possible to place the samples in their correct order of strength. A similar trial was made of two unknown samples of different leaves which had been extensively standardized in the laboratory. The results were compatible with the data supplied after the clinical trial. The study leads to the belief that clinical standardization cannot at present differentiate satisfactorily between digitalis preparations differing by much less than 25 per cent and to the position that close calculation of digitalis dosage is not necessary. The difficulties of clinical standardization are considered and a standard plan is drawn up.

*The Effect of Severe Anemia on the Heart and Circulation.* By LAURENCE B. ELLIS, Boston, Mass.

Cardiac failure and severe anemia frequently resemble each other in many respects in their symptomatology and physical findings. A study has therefore been made to determine the effects of severe anemia on the cardiovascular system. Forty-four patients were studied, and 26 of these were also examined when their anemia had improved. Thirty-three had dyspnea on exertion which disappeared in every case followed. Twenty showed dependent edema, and in all but 2 of the 10 followed it disappeared. One had typical anginal pain which ceased with relief of anemia. Twenty-eight patients had basal or apical systolic murmurs of varying intensity, and of the 21 followed these decreased or became absent in 11. One patient also showed a diastolic murmur which disappeared. Twelve of the 23 cases who were followed by serial teleroentgenograms showed a diminution in the cardiac size as the anemia lessened. In 6 the blood pressure increased definitely. Electrocardiograms were taken in every case and in 26 patients they were repeated following recovery. No serious abnormalities were present. In 5 there was an increase in the amplitude of the T waves and in 3 an increase in the amplitude of all complexes.

Severe anemia produces demonstrable changes in the heart and circulation, and these changes usually disappear with recovery from the anemia. Although some of the symptoms and signs which occur in severe anemia are similar to those occurring in heart disease and failure, there are certain manifestations of heart failure which are rarely if ever produced by anemia alone. These differential diagnostic signs are orthopnea, pulmonary or peripheral venous congestion, abnormal cardiac rhythms and marked electrocardiographic abnormalities.

*The Influence of Inorganic Salts on Salyrgan Diuresis.*

By MARSHALL N. FULTON and (by invitation) CLAYTON B. ETHRIDGE and DAN W. MYERS, Boston, Mass.

Studies have been made on normal dogs to determine the modifying effect of various inorganic salts on salyrgan diuretic action. To establish an approximately similar state of water and salt balance, the animals were maintained for one week prior to each test day on a constant food, sodium chloride and fluid intake. On the test days normal equivalent amounts of different salts were administered as a single dose per os, followed in two hours by an intravenous injection of salyrgan. Quantitative measurements of urine output and analyses for changes in blood and urine chlorides and plasma CO<sub>2</sub> combining power were made in each experiment. Control studies include comparable data on the action of salyrgan and of the various salts when given separately.

The results establish that acidifying salts plus salyrgan produce marked diuresis, whereas alkalinizing salts inhibit diuretic action. Neutral salts influence the diuretic action of salyrgan in the same manner as does water: a diuresis of moderate degree results. Acidifying salts working with salyrgan appear to show a syner-

gistic rather than a summative diuretic action of the two substances. The extent of diuresis from salyrgan following an inorganic salt seems to be influenced chiefly by the resultant alteration of acid-base equilibrium rather than the availability of particular ions for excretion.

*Observations on the Sulphur Content of Urinary Protein.*

By G. P. GRABFIELD, Boston, Mass.

The constitution of urinary protein was studied in various types of proteinuria. The method consisted in analyzing the urine for total nitrogen and total sulphur before and after the precipitation of the protein by heat and 0.5 per cent acetic acid. The differences between these two sets of analyses represent the nitrogen and sulphur in the urinary protein. That the figures so obtained are valid analyses of the protein has been checked by analyses of the protein obtained in relatively pure form by dialysis.

We shall go no further than to compare the relation of nitrogen to sulphur in the protein excreted in various types of proteinuria. The only significant variation was found in patients showing the nephrosis syndrome. In such patients the protein excreted is very low in sulphur. The N/S ratio of such protein is often as 200:1 as compared with about 20:1 in other types of albuminuria. There is considerable daily variation in the sulphur content of excreted protein. This fits in with the observations of K. Lang and E. G. Schenck who observed daily and even hourly variations in the amino-acid construction of the plasma proteins.

Other types of albuminuria studied were those of chronic glomerulonephritis, toxemia of pregnancy, orthostatic, exercise and cardiac decompensation. In addition, there were single cases of mercuric chloride poisoning and hyperglobulinemia.

The findings here reported suggest a fundamental alteration in the protein metabolism affecting the structure of protein in nephrosis. Similar studies on protein structure in other conditions might prove fruitful.

*The Frequency and Significance of Changes of the Expiratory Volume of the Chest during Routine Measurement of Basal Oxygen Consumption.* By JAS. A. GREENE, with technical assistance of MURIEL WARD, Iowa City, Iowa.

Many of the technical errors that may occur during the measurement of the basal oxygen consumption by the closed circuit method have been pointed out, but the effects of changes of the expiratory volume of the chest have not been generally appreciated. Benedict found that this factor was not significant in normal subjects, but in patients Greene and Coggeshall observed changes which materially altered the calculated results.

Significant changes of the expiratory volume of the chest were found to occur in 23.1 per cent of 2985 spiograms obtained during routine measurement of basal oxygen consumption, introducing an error ranging from 15 to as much as 45 per cent. This error may result in under-estimation as well as over-estimation of the actual

oxygen consumption. We shall discuss the conditions under which this error occurs and suggest a method of eliminating it.

*The Effects of the Introduction of Substances into the Cisterna Magna on the Blood Pressure of Dogs.* By H. RESNIK, C. PILCHER and F. M. MASON (by invitation) and T. R. HARRISON, Nashville, Tenn.

It is not yet known whether the several types of clinical hypertension are of nervous or of chemical origin. Most studies of pressor substances have been concerned with their action when administered intravenously or subcutaneously. Under such circumstances it is often difficult to distinguish between central and peripheral effects. In order to avoid this disadvantage, we have injected compounds into the cisterna magna and observed the blood pressure.

Various substances such as alkaloids, endocrine preparations, anaesthetics, other organic compounds and inorganic salts have been tested. Striking and prolonged pressor reactions have been observed from potassium and ammonium salts. When the cisterna is opened and the injection made caudad, effects are absent. Small amounts of these salts applied to the floor of the fourth ventricle cause a pronounced rise in blood pressure. Attempts to determine more exactly the site of action are in progress.

The pressor effects of potassium can be prevented by the previous administration of calcium. The injection of anions such as oxalate, phosphate and citrate, which diminish the ionization of calcium, results in prolonged rise in blood pressure.

Judging from the few cases so far studied, patients with uremia and phosphate retention have a diminution in the amount of ionized calcium in the spinal fluid, although the total calcium may be normal. The intracisternal administration of calcium salts has seemed to be beneficial in several patients with uremia.

*Hemoglobin Regeneration and Critical Changes in the Plasma Proteins.* By CLARK W. HEATH and (by invitation) F. H. L. TAYLOR, Boston, Mass.

Patients with severe anemia, who are regenerating blood, frequently show pitting edema and retention of fluids, unrelated to kidney dysfunction. A study of this phenomenon was made on the hypothesis that, since the red blood cells are rich in protein as compared to the blood plasma, the manufacture of red blood cells in the absence of any excess of protein in the diet may result in a reduction of the plasma proteins, thereby lowering the osmotic pressure of the blood.

The plasma proteins during blood regeneration were frequently reduced, even in the presence of a positive nitrogen balance. Edema appeared, however, only infrequently when the plasma proteins reached critical low levels. There was a great retention of nitrogen: the urine nitrogen was as low as 2 grams per day in some cases. Nevertheless, even in cases in which the nitrogen balance was negative, blood regeneration and a gain of total circulating nitrogen took place.

It was concluded that a reduction of the plasma osmotic pressure is a factor in the production of edema observed in anemia undergoing remission. The observations emphasize the increased protein requirements of the patient recovering from anemia.

*Visualization of Congenital Arteriovenous Fistulas of the Extremities by Arteriography.* By BAYARD T. HORTON and (by invitation) RALPH K. GHORMLEY, Rochester, Minn.

By means of arteriography with thorotrast the location and architecture of congenital arteriovenous fistulas of the extremities have been visualized and confirmed at surgical exploration. In the literature, prior to the use of arteriography, exploratory operations in attempts to close the fistulas had been followed by amputation of the involved extremity in 58 per cent of cases. Since the use of arteriography, where explorations have been carried out, amputation of the involved extremity in our experience has not been necessary in a single instance. Serial arteriograms illustrate the manner in which arteriovenous fistulas function. One can only interpret these properly when the pathologic physiology of a fistula is understood. The injected medium, like the blood, takes the course of least resistance, which is from the artery through the fistula to the veins. When the brachial or femoral artery is closed by pressure proximal to the point of injection of the thorotrast, there is always a leakage of the medium into the veins, which is invariably present when the first film is exposed. This is diagnostic of an arteriovenous fistula. The amount of filling on the venous side is proportional to the size of the fistula. If the fistula is large, the opaque medium will pass directly from the artery to the vein with little or no filling of the arterial tree distal to the site of the fistula. If the fistula is small, only a small amount of the opaque medium leaks into the veins. Enough remains in the arterial tree to visualize it completely distal to the site of the fistula. Detailed clinical and physiologic studies have been carried out in forty-one cases.

*Blood Velocity at Varying Levels of Metabolism in Subjects With or Without Thyroid Disease.* By J. W. MACY (by invitation), T. S. CLAIBORNE (by invitation) and L. M. HURXTHAL, Boston, Mass.

This study was begun to compare the blood velocity in patients with low metabolic rates without myxedema with the blood velocity in patients with myxedema. The study was then extended to include the following; normal individuals, patients with hypometabolism, myxedema, toxic goitre, toxic goitre with heart failure and heart failure without goitre. Over 120 subjects were tested by the decholin method.

The results show that low metabolic rates without myxedema give normal blood velocity rates. Some of these cases were due to proved pituitary deficiency, while in others the cause of the low B.M.R. was not determined. In myxedema, on the other hand, it was decreased. As would be expected increased blood velocity

rates were found in toxic goitre, but in patients with congestive heart failure and toxic goitre normal values were obtained. In patients with congestive heart failure without thyroid disease decreased rates were found. The normal values found in toxic goitre with heart failure were interpreted as being due to counteracting effects of hyperthyroidism on the one hand and heart failure on the other.

The results in the cases of low metabolism without myxedema are additional evidence that myxedema is not synonymous with hypometabolism and thyroid atrophy secondary to deficient thyrotropic hormone of the pituitary, a theory quite in vogue at present.

*Observations on the Character of the Pain Producing Substance in Contracting Skeletal Muscle.* By L. N. KATZ and (by invitation) E. LINDNER and H. LANDT, Chicago, Ill.

The following procedures were found to lessen the amount of exercise necessary to cause continuous pain in contracting muscles of normal subjects: (a) generalized anoxemia, (b) elevation of the CO<sub>2</sub> content in the blood contained in an ischemic limb, (c) trapping the blood coming from the exercised lower extremities in an ischemic limb. Alkalinization of the subject with large quantities of sodium bicarbonate definitely increases the amount of exercise required to produce pain.

The stimulus for pain, from these observations, appears to be the accumulation of a chemical metabolic substance (or substances) which diffuses readily into the blood stream and is altered quickly when the oxygen supply is adequate. It appears to be an acid, such as lactic or phosphoric acid, formed during the catabolism of muscular activity.

*Study of Total and Basal Metabolism in Obesity and Undernutrition.* By J. LERMAN and (by invitation) P. C. BAIRD, Boston, Mass.

The total metabolism of 6 underweight and 8 overweight patients was studied by determining their "insensible perspiration" according to the method of Benedict and Root and of Newburgh, slightly modified. The basal metabolism was studied simultaneously. Several of the patients were observed over long periods of time while undergoing changes in weight, i.e. gain in weight in the underweight group and loss of weight in the obese group.

A correspondence between the insensible weight loss at night and the basal metabolic rate, as observed by Benedict, was found also in 9 of our cases, but was doubtful in 2 and absent in 3.

There are two types of individuals represented in the undernutrition group:

*Type 1:* The total metabolism (insensible perspiration) in this type is low, being only slightly greater than the basal metabolism; the appetite is poor and the patients are lethargic. As they gain weight, on an increased diet, the total metabolism rises 50 per cent or more. This is not in any significant degree due to increase in surface area or specific dynamic action of food.

*Type 2:* The total metabolism in this type is high before treatment. The patients have a good appetite but are nervous and overactive. As they gain weight, total metabolism increases, but to a less extent than in Type 1. The basal metabolism is about the same in the two types, being about minus 10 in each.

The effect of insulin (commercial and crystalline) in undernutrition was observed. It does not produce any change in total or basal metabolism nor does it affect the rate of weight gain on a constant diet. Its effect seems to be solely to increase appetite.

In obesity the total metabolism is about the same as in the second type of undernutrition. Consequently the caloric output per square meter of surface area is very low. On a reduction diet (1,100 to 1,300 calories) there is always a progressive loss of weight. As the patient loses weight the insensible perspiration diminishes, but the basal metabolism usually does not change much.

*Malignant Hypertension: The Pre-renal Phase.* By CLIFFORD WILSON and PAUL KIMMELSTEEL (introduced by Edwin A. Locke), Boston, Mass.

A study of the clinical and histological features of so-called benign and malignant hypertension has led to the conclusion that cases of malignant hypertension occur without kidney involvement. Such cases lead to a clearer conception of the disease entity than is obtained from the classical description of Volhard and Fahr.

We believe that the condition commonly known as malignant nephrosclerosis represents only an end stage of the disease. The clinical picture is that of unusually high blood pressure occurring in young subjects and terminating in renal failure. Histologically they show characteristic arterial lesions, namely, productive endarteritis and necrotizing arteriolitis in the kidney and other organs, associated with focal glomerulonephritis.

Certain cases are here described in which death occurs without renal involvement. From a consideration of the clinical and histological findings we feel that these cases can be separated from the group of benign hypertension. Clinically they show unusually high blood pressure developing in young subjects. Death occurs from extra-renal causes but has a more fulminating character than is seen in benign hypertension. Usually with cerebral manifestations, such as have been termed hypertensive encephalopathy. The characteristic arterial lesions of malignant hypertension are usually present in these cases though less prominent than in the classical picture described by Fahr. We conclude from this clinical and histological study that malignant hypertension is a primary vascular disease in which the renal manifestations occur as a terminal event.

*Observations on Associated Tuberculous and Non-tuberculous Pulmonary Lesions.* By F. MAURICE MCPHEDRAN, Philadelphia, Pa.

The association of non-tuberculous bronchopneumonia with various tuberculous pulmonary lesions has been ob-

served. Tuberculous lesions have their base on the pleura and rarely invade the cardiophrenic area, where non-tuberculous lesions originate and spread along the diaphragm. Extensive pleural effusion and thickening are rare when either of these lesions occurs alone but are common with contiguous tuberculous and non-tuberculous infiltrations. Unusually numerous wide-spread foci of calcification are seen as they heal. The tuberculous element of these associated lesions, even when excavated, tends to heal rapidly and permanently. The non-tuberculous lesion may persist for years as a relapsing bronchopneumonia with persistently negative sputum.

Non-tuberculous bronchopneumonia has been observed in a few cases in association with a uniformly distributed miliary tuberculosis. Of these, three are apparently well except for a persisting bronchopneumonia. The fourth died of a descending apical-caudal tuberculosis after clearing the miliary lesion (postmortem). In all four cases the tuberculous pulmonary lymph nodes were enormously enlarged. In two, non-tuberculous bronchopneumonia and tuberculous nodes were observed before the development of miliary tuberculosis. In all these, and in some other cases, serial observations suggest that the non-tuberculous bronchopneumonia caused hematogenous spread by flooding the nodes rather than by "depressing resistance."

*Effects of Alpha-Dinitrophenol on Energy Metabolism, Blood Chemistry and Renal Function: Studies in Obese Subjects.* By MAURICE BRUGER, CAMERON V. BAILEY and GEORGE C. THOSTESON (introduced by H. O. Mosenthal), New York, N. Y.

Nine obese women were studied under basal conditions and every hour for three or four hours after the ingestion of alpha-dinitrophenol (3.5 mgs. per kilogram). The respiratory exchange was determined by a modified Tissot method. In the blood sugar, lactic acid, acetone bodies, urea nitrogen, nonprotein nitrogen, free and ester cholesterol and chlorides were determined. In the urine urea nitrogen and nonprotein nitrogen were estimated. The basal metabolic rate, the combustion of protein, fat and carbohydrate, the urea ratio and the urea clearance were obtained by calculation from the above data.

The augmented metabolism after dinitrophenol was accompanied by small but appreciable increases in mouth temperature, respiration and pulse rate; the fuel mixture was definitely altered; carbohydrate and fat combustion were increased while protein catabolism was significantly diminished. Appreciable quantities of carbohydrate were burned, even though under control conditions carbohydrate was absent from the fuel mixture because of previous dietary restrictions.

The blood sugar, lactic acid and acetone bodies were increased; the blood chlorides and urinary nitrogen were decreased; the blood urea nitrogen, nonprotein nitrogen, free and ester cholesterol remained practically unchanged. The urea ratio and urea clearance showed little or no change in renal function.

*Further Observations on the Leucocytic Response Induced by the Intramuscular Injection of Liver Extract.* By JOHN H. POWERS, with the assistance of CYNTHIA VANDOREN, Cooperstown, N. Y.

Observations on the degree and character of the leucocytic response induced by the intramuscular administration of liver extract to 7 normal subjects and to 3 surgical patients with infection have been described and presented in graphic form.

The normal individuals were divided into two groups. To the first of these 3 cc. of liver extract were given at 9 o'clock in the morning. The leucocytic response was neutrophilic in character, reached its peak in 6 hours and was composed of a relatively greater increase (235 per cent) in the young forms than in the mature cells (96 per cent). The number of juveniles increased from zero to 270 per cu. mm.

There were 3 normal subjects in the second group who received the same amount of extract at 5 o'clock in the morning. The total white cells doubled, the neutrophils increased 169 per cent and the band forms, 370 per cent. The juveniles rose from zero to 400 per cu. mm. The peak of the rise again occurred 6 hours after the injection. The results suggest that the leucocytosis induced by the intramuscular administration of liver extract to normal individuals is due either to direct or indirect stimulation of the bone marrow.

Three surgical patients with sepsis were given liver extract intramuscularly. In each case the leucocytic count was low before the injections were started, and a well marked leucocytosis occurred thereafter. The response was entirely neutrophilic in character.

*A Study of the Calcium, Phosphorus, and Energy Exchange in a case of "Pituitary Basophilism."* By R. H. FREYBERG (by invitation), L. H. NEWBURGH, PAUL S. BARKER, R. L. GRANT (by invitation) and FREDRICK A. COLLIER (by invitation), Ann Arbor, Mich.

The calcium, phosphorus and energy exchange was studied over a long period of time in a 19-year-old male, who clinically exhibited the syndrome of pituitary basophilism (with marked skeletal demineralization and obesity) and at autopsy showed an anterior-lobe pituitary adenoma of incompletely differentiated basophil cell type.

The serum calcium was slightly elevated, the serum phosphorus distinctly low. On a low calcium diet the urinary calcium was rather high, and the calcium balance negative. When the diet was made rich in calcium the urinary calcium remained unchanged, the fecal calcium became great, and the balance remained slightly negative. Administration of large amounts of  $\text{CaHPO}_4$  was attended by huge fecal excretion of calcium, unchanged urinary calcium and only a slight retention of calcium. Irradiated ergosterol, haliver oil, yeast,  $\text{NH}_4\text{Cl}$ ,  $\text{HCl}$  and  $\text{CaCl}_2$  were of little or no benefit. When calcium gluconate was injected intravenously much of the calcium was retained with sufficient phosphorus to form  $\text{Ca}_3(\text{PO}_4)_2$ . Study of the calcium and phosphorus exchange

did not indicate abnormal parathyroid function but showed insufficient absorption of calcium.

Undernutrition caused weight loss exactly as predicted for normals, thus indicating normal energy exchange and the absence of any unusual metabolic feature as the cause of this patient's obesity.

*The Response of the Systolic Blood Pressure to Cold and to Mental Stress in Essential Hypertension, Duodenal Ulcer, Vasomotor Instability, Psychoneurosis and Normal Controls.* By ROBERT STERLING PALMER, Boston, Mass.

The systolic blood pressure in the left brachial artery has been studied, noting spontaneous variations, response to mental stress and response following immersion of the opposite hand in cold water for one minute. Patients with hypertension, patients with duodenal ulcer, apparently normal people with some signs or symptoms of vasomotor instability, and entirely normal controls have been compared. Spontaneous variations are greater in patients with essential hypertension than in normal controls, in patients with functional nervous disorders or in patients with duodenal ulcers. Seventy-six experiments on twenty-three patients with essential hypertension show that the response of the systolic blood pressure to mental stress, while not quite as great as, is, nevertheless, comparable to the response to cold. Both are greater than in entirely normal controls. Patients with duodenal ulcer, patients with functional nervous disorders (functional indigestion, attempted suicide, psychoneurotic vomiting) and patients with vasomotor instability commonly show a marked response of the systolic blood pressure to cold but comparatively little to mental stress. Entirely normal controls appear to have a moderate, but relatively greater, response to mental stress compared to the response to cold. Observations on one case of coarctation of the aorta and on one case of cortical adenoma show a marked response to cold but a relatively smaller response to mental stress.

It is suggested: (1) that immersion of the hand in cold water is a potent stimulus to the autonomic nervous system; (2) that the degree of systolic blood pressure response may indicate the irritability of the autonomic nervous system as an unconditioned response; and (3) that a characteristic of essential hypertension is not an unduly irritable autonomic nervous system, such as is found also in cases of vasomotor instability, functional nervous disorders and duodenal ulcer, but that the vascular response is especially conditioned to mental stress.

*Clinical Glycosuria Associated with Biliary Obstruction.* By HERBERT POLLACK and (by invitation) HERMAN LANDE, New York, N. Y.

The experimental investigations of the functions of the liver have demonstrated the importance of this organ in the carbohydrate cycle. In hepatectomized animals the injection of glucose at a rate greater than its immediate usefulness to the organism produces hyperglycemia and



glycosuria. When insufficient glucose is supplied hypoglycemia occurs.

The application of these physiological concepts to clinical medicine is demonstrated in the following case histories. The first patient had colicky epigastric pains for one month and acholic stools the last five days. Forty-five units of insulin were required with a diet of 160 grams carbohydrate to control glycosuria. Cholecystectomy and removal of stones from the common duct were carried out. Severe postoperative hypoglycemia followed. Glucose tolerance was normal three weeks later. Case number 2 was given 90 units of insulin with a diet of 250 grams of carbohydrate. Four weeks after cholecystectomy glucose tolerance was normal. Case number 3 was that of a man suffering from a carcinoma of the head of the pancreas with common duct obstruction. Ninety units of insulin were required with a diet of 200 grams carbohydrate to prevent glycosuria. Obstruction was relieved by cholecystgastrostomy. Post-operative hypoglycemia followed in spite of continuous intravenous glucose infusion. The icterus cleared up. Pancreatic obstruction increased, as evidenced by rising blood amylase. Glucose tolerance test was normal three weeks later.

*The Effect of Prolonged Dietary Restriction on Patients With Cardiac Weakness.* By S. H. PROGER and (by invitation) H. MAGENDANTZ, Boston, Mass.

Physiologists have demonstrated that prolonged dietary restriction (loss of approximately 10 per cent body weight in about six weeks) commonly produces the following results: Striking lowering of oxygen consumption (about 30 per cent), significant slowing of the pulse rate, lowering of blood pressure and lowering of fluid intake and output. If such changes occur without apparent harmful effects then their production seems eminently desirable in patients with cardiac weakness. We have, therefore, studied the effect on such patients, of dietary restriction with a loss of 10 to 15 per cent in body weight over four to six weeks after adequate preliminary control. The following features were studied: oxygen consumption, respiratory quotient, pulse rate, blood pressure, velocity of blood flow, cardiac output, venous pressure, vital capacity, respiratory rate, respiratory minute volume, blood cholesterol, blood glucose tolerance, fluid intake and output, nitrogen intake and output, chloride excretion, response of pulse and respiration to exercise on bicycle ergometer and incidental clinical changes. Daily observations were made over a period of eight to ten weeks, thereafter at two to four week intervals.

In four patients (3 normal weight and one underweight) thus far completed the results showed that a condition can be thus artificially produced in which the work of the heart approaches a minimum. Among other things there was distinct lowering of caloric and water exchange, drop in blood pressure, pulse rate, and cardiac output and increase in vital capacity and capacity for work.

If it is self-evident that the weight of an obese patient with cardiac weakness should be reduced, it appears just as self-evident that in patients with heart disease of normal and even low weight it should be reduced, since the theoretic as well as actual benefits are even more striking in the latter group.

*Studies on Microbic Dissociation of Micrococcus Tetragenus Obtained From a Nonfatal Case with Septicemia, Purulent Arthritis and Meningitis.* By HOBART A. REIMANN, Minneapolis, Minn.

The organism when first isolated grew in clusters and formed white colonies resembling a slowly growing staphylococcus. Upon aging on agar the colonies became yellow, developed yellow daughter and white daughter colonies, and later translucent colonies appeared. These colony forms seemed to correspond with the "M," "S" and "R" phases involved in microbial dissociation of other organisms. But later a shell-pink and an orange-brown form were also induced. Cocci from the yellow colony formed large tetrads, the white formed tetrads or clusters, and the translucent colony cocci were minute and markedly pleomorphic. The 3 forms would ordinarily be mistaken for different, unrelated organisms except that reversion from one form to another could be induced by appropriate methods in vitro and in vivo. Organisms from the yellow, white and translucent colonies differed sharply in their biological reactions as regards sugar fermentation, gelatin liquefaction, pigment production, hemolysis and resistance to numerous adverse conditions. The pink and brown forms behaved generally like the white. The five dissociant forms characterized by such different morphologic characteristics provide a unique opportunity for observations in dissociative phenomena. The pigments of the yellow, pink and brown colonies belonged to the carotinoid group and appeared to be chemical isomers, differing from one another spectroscopically.

Immune sera prepared with the 5 varieties were specific for the respective homologous variety. The sera agglutinated occasional strains from other sources indicating the existence of type specificity as well. Surprisingly, the sera prepared from the dissociant forms agglutinated specifically the corresponding dissociants of the type-specific strains from other sources.

*Reticulocytosis In Non-Hemolytic Jaundice.* By LEON SCHIFF and (by invitation) MURRAY L. RICH, Cincinnati, Ohio.

In a series of over thirty-five cases of regurgitation jaundice (obstructive and toxic or infective hepatic types) a moderate persistent reticulocytosis was frequently observed. In an attempt to explain the underlying mechanism intravenous injections of bilirubin and bile salts (decholin) and intramuscular injections of blood from jaundiced individuals were given to normals and to patients with anemia and the hematological effects studied.

*The Prognostic Significance of the Icteric Index in Lobar Pneumonia.* By GERALD S. SHIMLEY and (by invitation) W. W. WANDELL, Cleveland, Ohio.

Considerable difference of opinion exists in regard to the prognostic significance of jaundice in pneumonia. Although many clinical observers have felt that jaundice is a grave finding, the few references to be found in the literature are equivocal. In the present study, which has included nearly 200 cases of lobar pneumonia, the relationship of the icteric index to mortality has been investigated. Our results show that the death rate is proportional to the height of the icteric index. If the index is under 10, the mortality rate is slightly below 30 per cent; if over, it is approximately 60 per cent. Such factors as type of pneumococcus, infection of blood stream, or age do not influence this graver prognostic significance of the higher indices. The definite value of specific serum therapy in Type I infections is brought out by the fact that although the death rate in untreated cases, with an index of 10 or over, was 57 per cent, it was only 10 per cent in such cases receiving serum.

*Iron Retention in Hypochromic Anemia.* By W. M. FOWLER (by invitation), ADELAIDE BARER (by invitation) and FRED M. SMITH, Iowa City, Iowa.

Studies of iron balance have been carried out in forty patients in an attempt to ascertain the amount of iron retained and the factors influencing the retention of iron and formation of hemoglobin in hypochromic anemia.

Those patients whose anemia was idiopathic in origin and those in whom it was secondary to chronic blood loss showed no appreciable difference in retention of iron.

With a normal iron intake (12 mgm. per day derived from food) less iron was retained by those patients with an achlorhydria than by those with a normal or low gastric acidity. The administration of HCl and pepsin did not increase the retention of iron under these conditions.

With the administration of iron (500 mgm. per day) in the form of ferric ammonium citrate a surprisingly large amount was retained. This retention was not appreciably influenced by achlorhydria nor by the administration of HCl.

The addition of a small amount of copper to the medicinal iron seemed to decrease the amount of iron retained, although the hemoglobin regeneration was more rapid and the percentage of the iron that was utilized as hemoglobin was greater.

*The Influence of Castration on Stimulation of the Thyroid by Pituitary Thyrotropic Hormone.* By PAUL STARR, Chicago, Ill.

During studies of pituitary thyrotropic action in guinea-pigs (begun in 1931 and reported in 1932 and 1934), a fairly uniform response in the form of elevated metabolic rates has been found after daily subcutaneous injections of 0.1 cc. of a pituitary solution prepared by Parke, Davis and Company. In a group of seven female animals, after the fifth daily injection the basal metabolic rate ranged from +35 to +63 per cent.

In five female animals previously castrated, after the fifth daily injection the metabolic rate ranged from -3 to +17 per cent. In four other animals the rate rose rapidly but the rise was delayed in onset. In one animal castrated after 4 daily injections, having a metabolic rate elevated +35 per cent, the rate dropped during continued injections to  $\pm 0$  per cent five days after the operation; while the control, continuously injected animal continued to have an elevated rate of +45 per cent for 23 days and then declined as has previously been shown.

Histologic study of the uncastrated animals shows the usual marked hyperplasia of the thyroid. The thyroids of the castrated animals similarly treated show no hyperplasia, or a peculiar engorgement is found if the metabolic rate has risen. An attempt to correlate this prevention of the thyrotropic action with release of gonadotropic pituitary hormone by castration is being made by assays of the blood. In a normal man weighing 175 pounds the same solution raised the metabolic rate in a few days from -5 to +15 per cent. In a castrated woman weighing 93 pounds similar dosage raised the rate from +2 to +7 per cent in two weeks.

Extension of these studies in other animals and humans is being carried on intensively. It is thought that this is a demonstration of an endocrine thyroid protective or inhibiting mechanism.

*The Effect of Vitamin B upon Hematopoiesis in the Rat.*

By FRANK H. BETHELL (by invitation) and CYRUS C. STURGIS, Ann Arbor, Mich.

The prevention and cure of milk anemia of the rat does not depend exclusively upon the mineral content of the diet. A supplementary action to iron and copper is exerted by the vitamin B complex. This property, under the conditions of the present studies, is not possessed by either vitamins B<sub>1</sub> or B<sub>2</sub> but is contained within the B<sub>6</sub> fraction.

One of the earliest changes observed during treatment with iron and copper supplements is a transition of the anemia from a microcytic to a macrocytic type. Upon the addition of the vitamin B<sub>6</sub> fraction the mean erythrocyte diameter rapidly becomes normal. When supplementary B<sub>6</sub> fraction together with iron and copper is given throughout the course of treatment, temporary macrocytosis does not occur.

In the cure of the milk anemia of the rat vitamin B<sub>6</sub> fraction apparently exerts an influence both upon the synthesis of hemoglobin and the maturation of the erythrocyte.

*The Development of Pneumococcal Antibodies in Children.* By W. D. SUTLIFF and (by invitation) JOHN A. V. DAVIES, Chicago, Ill.

The appearance of serum immune bodies and other immune reactions with increasing age, without apparent infection by the corresponding microorganisms, has given rise to two hypotheses: (1) subclinical infection and (2) physiological development. The first of these, or contact between host and microorganism without disease,



may be investigated in man during a period when immune phenomena are being established, and this has been done in the study to be reported. The study is facilitated by the ready identification of type specific pneumococcic strains and their corresponding antibodies, and knowledge that such antibodies develop most often about the age of 2 years. Contacts with pneumococci and the appearance of blood bactericidal power for certain strains were observed in 9 infants from 5 to 21 months of age during a period of 5 winter months. Three infants developed antibodies for Type II pneumococci. None were observed to carry Type II pneumococci in their pharynges during this period. Data were assembled as to the health of all the infants and as to the incidence, type and pathogenicity of the pneumococcic strains that were encountered. It is concluded that neither true infection nor a carrier state of as long as one week are necessary to the appearance of Type II pneumococcic bactericidal action of the blood.

*The Effect of Iodine on Certain Aspects of Cholesterol Metabolism.* By KENNETH B. TURNER and (by invitation) FREDERICK H. SHILLITO, New York, N. Y.

Thoracic duct fistulae were produced in otherwise normal dogs and the chyle cholesterol determined after feeding cream and cream plus cholesterol. Thorough iodization of another group of dogs did not prevent the rise normally observed in chyle cholesterol after ingestion of cream and cholesterol. It was concluded that KI had no effect upon the absorption of cholesterol.

KI was given to rabbits previously fed with cholesterol over a long period and showing a markedly elevated cholesterol in the blood. A sharp rise in blood cholesterol occurred that disappeared when KI was stopped. This rise took place regardless of the presence or absence of the thyroids. The previously reported action of KI in preventing a rise in blood cholesterol when fed concurrently with cholesterol was shown to be of a transient nature.

An attempt was made to determine whether KI was capable of mobilizing tissue cholesterol. Rabbits were fed cholesterol for several months. One-third of the animals were killed and the liver cholesterol determined. Of the surviving rabbits half were given KI for a further period, and the other half used as controls. After two months all the animals were killed and the liver cholesterol determined.

*The Phase Angle as a Test for Thyroid Dysfunction.* By FRANKLIN D. JOHNSTON (introduced by Frank N. Wilson), Ann Arbor, Mich.

Following the method described by Brazier,<sup>1, 2</sup> we have measured the phase angle of a number of normal individuals and patients having hyperthyroidism or myxedema.

For normal adult males and females the phase angles

<sup>1</sup> M. A. B. Brazier. *Institution of Elect. Engineers*, 1933, 73: 204.

<sup>2</sup> M. A. B. Brazier. *Lancet*, 225: 742, Sept. 30, 1933.

fall into two distinct groups according to sex. For each sex there is considerable individual variation from the mean of the group, but the mean values agree closely with the results of Brazier. Patients with hyperthyroidism show a definite decrease in the phase angle usually outside the limits of normal variation and occasionally nearly 50 per cent below the average normal value for the patient's sex. Following thyroidectomy there appears to be no change in the phase angle until a period of approximately three weeks has elapsed. After this time, the angle increases gradually until it is within normal limits. After oral administration of thyroid substance no lowering of the phase angle has been observed until the patient has been taking the drug for at least a month. The phase angles obtained from a few patients with myxedema, before thyroid therapy was instituted, were within normal limits.

*The Occurrence of Coronary Air Embolism in Artificial Pneumothorax.* By T. M. DURANT<sup>1</sup> (introduced by Frank N. Wilson), Ann Arbor, Michigan.

This paper is based on the observation of a case in which, following air embolism complicating attempted artificial pneumothorax, a series of exceedingly interesting electrocardiographic changes was observed. The case was predominantly one of unilateral pulmonary tuberculosis and congenital pulmonary stenosis, in which an electrocardiogram prior to treatment had been negative except for right axis deviation. A series of electrocardiograms, following the acute collapse due to the air embolism, showed findings and progressive changes in both the initial and final deflections of the ventricular complex typical of myocardial infarction. The first curve was taken within three hours of the accidents. The changes noted persisted for approximately a year, at the end of which time there was a return to the type of curve observed before the infarction.

The general subject of air embolism is discussed, and reasons are given for attributing the incident described to involvement of the coronary vessels in the embolic process.

*A Sphygmographic Method for the Study of Systolic and Diastolic Blood Pressure in Dogs: Illustrated with Curves from Normal and Hypertensive Animals.* By J. EDWIN WOOD, JR. and (by invitation) JAMES CASH, University, Virginia.

Methods for the study of systolic and diastolic blood pressure in the unanesthetized dog have been generally unsatisfactory. With one exception (Cash) long time experiments showing both systolic and diastolic values are not available in the literature. The purpose of this paper is to describe a modification of the Kolls-Cash method which permits repeated systolic and diastolic readings on the unanesthetized dog and to illustrate the procedure with curves made up of numerous determinations on normal and hypertensive animals.

<sup>1</sup> Department of Internal Medicine, University of Michigan Medical School.

A compound lever of special design (Kolls) is arranged with a mercury column, a pneumatic cuff for the dog's leg, and a brass control valve as in the usual sphygmographic assembly. The original Erlanger rubber bulb is difficult to obtain and the more recent Erlanger sphygmoscope has been tried and found to be unsatisfactory in the lower diastolic ranges. These difficulties have been obviated by the use of a metal pressure bell carrying a heavy piece of automobile tubing as membrane between the cuff and lever systems. This simple modification allows sensitive and accurate response of the compound lever at all blood pressure levels. The accuracy of the method is supported by the original maximum-minimum valve experiments of Kolls and Cash as well as by our own recent observations. A simple check with simultaneous auscultatory and sphygmographic records on humans is convincing.

Eight dogs have been subjected to several procedures with repeated blood pressure observations before and after operation. In addition to the blood pressures, twenty-four hour water intake, output and specific gravity and blood nonprotein nitrogen determinations have been recorded at intervals.

The following table indicates operative procedure, duration of study and result. From 100 to 160 blood pressure determinations have been made on each animal.

Dog	Operation	Duration of study	Before operation		After operation	
			Mean systolic	Mean diastolic	Mean systolic	Mean diastolic
		<i>Months</i>				
C-1	Goldblatt clamps	7	167.4	71.9	200.0	111.3
C-15	Goldblatt clamps	13	160.9	52.85	185.4	79.95
C-9	Goldblatt clamps	13	148.0	54.0	215.3	96.4
C-11	Subtotal					
	nephrectomy	15	175.1	63.5	186.4	76.3
C-2	Subtotal					
	nephrectomy	15	172.2	79.3	179.8	87.6
C-6	Partial renal					
	artery ligation	15	160.0	61.2	193.7	79.2
C-8	Partial renal					
	artery ligation	15	132.9	60.9	133.7	66.9
C-3	Partial renal					
	artery ligation	15	150.5	55.3	198.0	87.1

Significant elevations in systolic and diastolic blood pressure have occurred in six of the eight animals. Diastolic readings have been less variable than systolic and are essential to the study of hypertension in dogs. The most striking blood pressure rise has taken place when renal ischemia has been produced by the application of Goldblatt clamps to the renal arteries.



# A STUDY OF THE STANDARDIZATION OF DIGITALIS. I. A METHOD FOR CLINICAL STANDARDIZATION

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It was at least partly because clinical observations led to the conclusion that different specimens of digitalis varied in their potency that the need for assay of the drug was realized. It would seem natural, therefore, to suppose that much attention would have been given to the clinical standardization of digitalis. In an exact sense, however, this is not the case. The problem is, indeed, a difficult one. It is necessary to know the rate of absorption of digitalis, and desirable to know its fate and its rate of elimination. The rates of absorption and elimination of some preparations have been worked out, though not in any large series or with great accuracy; the results show considerable variation from person to person, so that the averages reached stand for a wide range in a relatively small group of cases. When the drug is given by mouth, the speed and amount of absorption varies with different preparations and perhaps with the degree of congestive failure, if that condition is present. The tincture, however, is apparently fairly promptly and evenly absorbed in most cases (1). Intravenous or subcutaneous administration eliminates certain variables, but is restricted to certain preparations and is not commonly necessary in clinical practice. Very little, indeed, is known of what becomes of the digitalis that is absorbed. Some effect of a full dose may last as long as three weeks (2). The Eggleston method (3) of giving the drug involves the calculation of the amount required for an individual on the basis of his weight. This plan coupled with the average daily excretion found by Pardee (4) has been widely used; it assumes the possibility of clinical standardization.

Clinical experience with the body-weight method of calculating the dose of digitalis gives variable results, even with the same sample of the drug. This is not astounding, since two patients in exactly the same condition can hardly be found. The weight of the body is but one of its variable

functions. The number of its functions which influence the effect of digitalis must be great. It has been shown that age is important, for young children require up to two or two and a half times the body-weight dose for adults (5). The Eggleston method (3) of giving the drug has been found very useful, and has done much to clarify the problems of the clinical use of digitalis.

It is remarkable that we have not been able to find any report of the clinical standardization of digitalis in which effort was made to study the limits of accuracy of the methods employed, by giving the same preparation in different strengths to the same individual. The most interesting attempts at exact clinical comparison of different preparations are those of Gilchrist and Lyon (6, 7) and Martin (8). Both of these reports are based on the study of three samples of leaves supplied by the Hygiene Committee of the League of Nations. The samples were extensively "standardized" by both frog and cat methods in many laboratories (9). Gilchrist and Lyon finally restricted their considerations to observations made on patients with auricular fibrillation and adopted as their criterion for the effect of the drug the drop in ventricular rate in terms of the initial ventricular rate. Martin employed patients with cardiovascular disease, both with and without auricular fibrillation, and took into consideration clinical evidence of improvement, minor toxic symptoms and pulse rate, blood pressure, and A-V conduction time. In both of these groups there was a variable degree of heart failure. In neither was special effort made to give all samples to the same individuals. Briefly summarized, the potencies of leaves "A" and "B" are given in terms of leaf "C." It should be pointed out that with improved understanding of the problem and with better standardization of technique, more consistent results can readily be attained. The two clinical studies reversed the position of leaf "B" with respect to "C." That the results are

so divergent is, of course, due in large part to the fact that various methods and techniques were used. The divergence illustrates the difficulties involved.

TABLE I  
*Comparison of assays from literature*

Assay	Leaf	A	B	C
Frog methods, averages (9).....		50.1	94.3	100
Cat methods, averages (9).....		60.5	116.7	100
Clinical (6).....		64.3	88.9	100
Clinical (8).....		75.8	141.5	100

In consideration of the desirability of accuracy, purely clinical criteria seem out of the question for these tests. The degree of congestive failure changes spontaneously and with time. The clinical effects of digitalis seem to vary with the degree of failure. The occurrence of congestive failure is, furthermore, a general indication for digitalis treatment, so that it is difficult to withhold the drug in its presence. The long interval which must elapse to secure freedom from previous doses renders it impossible to reproduce the same conditions in successive tests. It seems best to eliminate, therefore, the factor of clinical congestive failure, as far as possible.

The results of Gilchrist and Lyon suggest that the drop in ventricular rate, in the presence of auricular fibrillation, expressed in terms of the initial ventricular rate, may be a good criterion for the effect of digitalis. This method apparently cannot be used for the trial of different samples in the same individuals, however, because the effect is demonstrably different at different times. (Is this because of variation in the degree of congestive failure (16)?) The method must be confined to statistical studies in which large numbers of individuals preferably without failure, not available to us, are employed. (It is well known that the effect of digitalis on the rate of the normal mechanism is too variable to be applied for the purpose in hand.)

#### METHODS

In planning our investigation, it was decided to rely principally and finally on objective observations, and to give to the same subjects all the samples of digitalis to be compared. The subjects

selected were normal individuals or patients with cardiovascular disease, but without clinically demonstrable congestive failure. They were studied by the usual clinical methods and were under observation for considerable periods before the tests began. Except in one instance (19 days), subjects were without digitalis for three weeks, and in many cases for much longer periods before each trial. No patient had significant fever, and no other important drug was given at the time of the tests.

The samples of digitalis were supplied by the Department of Pharmacology, in the form of tinctures, the strengths of which were unknown to us until the tests were completed. Because it was not our intention to compare the effects in different patients, their weights were not taken into consideration, although they were recorded; in no case was there important change in an individual's weight between trials. In each series each subject received the same dose of the preparations given him, except in one case. In the morning, after preliminary observations, 8 cc. (in the second series 10 cc.) were given. In the first and second series this was followed by three doses of 2 cc. each at 4-hour intervals; in the third series by a single dose of 2 cc. after 4 hours. This plan was adopted in the hope of detecting early differences among the effects of the samples. (The results show that a single dose is preferable.) The order in which the samples were given to patients was varied, but this variation does not seem necessary.

The special observations made included weight, height, blood pressure, and electrocardiograms. Symptoms of minor intoxication were carefully noted. In a few cases teleroentgenograms were made; although in some cases interesting changes occurred, the number of observations is not sufficient to justify their consideration at present. The present observations were made the day before the tests, immediately before the first dose, at 4-hour intervals during the day of the trial, and on the morning after (24 hours after the first dose). The usual leads of the electrocardiograms were taken with the subjects lying down at rest. The resistance was always satisfactorily low. The string deflection was standardized in the usual manner, and the standard deflection was recorded with each lead. The study of the electrocardiogram

grams comprised measurement of the cycle length, the "A-V" conduction time ("P-R" interval), the duration of "electrical systole" ("Q-T"), the amplitude and direction of the T wave, and observation of the form of other waves. Measurements were made with calipers under a magnifying glass, in most cases in Lead II, but always in the same lead for different samples of digitalis in the same individual. Some of the measurements are difficult and perhaps unreliable in the presence of auricular fibrillation. The few results from cases of fibrillation which we present are averages of ten consecutive cycles.

## RESULTS

A survey of the features observed convinced us that it seemed impossible to use most of them in any exact way for the comparison of the different samples of digitalis. Most of the usual

effects of moderate doses occurred. Symptoms were encountered only in a very few instances. The blood pressure, as may be expected, showed no significant changes. Slowing of the rate, though slight in many cases, occurred in 38 out of 44 observations, but was irregular. The "A-V" conduction time was usually lengthened, often very slightly. The T wave was often decreased in amplitude but sometimes not. No other important changes in the form of the deflections were seen.

It has long been known that digitalis shortens the duration of systole in experimental animals. Cheer (13) and Berliner (14) first called attention directly to the effect on the "Q-T" interval in human electrocardiograms. This interval is apparently a function of the cycle length (which stretches from end of one "T" to end of the next "T," and includes the "Q-T" interval to

TABLE II

*Electrocardiographic measurements and constant "K" immediately before and 24 hours after giving samples of digitalis of unknown potencies*

Sub- ject*	Digitalis** sample	P-R interval		T wave		Cycle length		Q-T interval		Constant "K"†	
		Before	After	Before	After	Before	After	Before	After	Before	After
1 Normal heart	A (3)	0.150	0.160	3.0	2.5	0.720	0.860	0.320	0.325	0.377	0.350
	B (2)	0.140	0.160	3.0	2.0	0.920	0.960	0.365	0.310	0.381	0.316‡
	C (1)	0.140	0.160	3.4	2.5	0.915	0.800	0.360	0.315	0.376	0.352
2 Luetic CVD	A (1)	0.170	0.180	-3.5	-3.0	1.280	1.010	0.480	0.410	0.424	0.408
	B (2)	0.170	0.180	-1.5	-1.7	1.140	1.390	0.440	0.420	0.412	0.356
	C (3)	0.170	0.180	-1.0	-1.5	1.070	1.245	0.410	0.400	0.397	0.358
3 AS CVD AF	A (1)	?	?	3.5	2.0	0.650	0.910	0.320	0.370	0.397	0.388
	B (2)	?	?	4.0	1.5	0.690	1.360	0.350	0.400	0.421	0.343‡
	C (3)	?	?	3.0	2.0	0.600	1.030	0.320	0.365	0.413	0.360
4 Normal heart	A (1)	0.140	0.150	3.0	2.0	0.745	0.645	0.325	0.300	0.377	0.374
	B (2)	0.130	0.170	3.5	2.0	0.705	0.750	0.335	0.318	0.399	0.367
	C (3)	0.145	0.160	4.0	2.0	0.740	0.730	0.335	0.310	0.389	0.363
5 Hy CVD	B (2)	0.160	0.180	1.6	1.4	0.805	0.860	0.355	0.340	0.396	0.367
	C (1)	0.150	0.160	1.0	1.0	0.780	0.820	0.350	0.350	0.396	0.387
6 Rh CVD	A	0.165	0.170	1.3	1.5	0.800	0.850	0.330	0.325	0.369	0.353
7 Rh CVD	B	0.195	0.210	1.4	1.3	0.625	1.200	0.320	0.365	0.405	0.333
8 Hy CVD AF	C	?	?	1.8	1.3	0.560	0.900	0.310	0.330	0.414	0.348

\* "CVD" is cardiovascular disease; "AS" is arteriosclerotic; "Hy" is hypertensive; "Rh" is rheumatic; "AF" is auricular fibrillation.

\*\* "A," "B" and "C" are dilutions of a standard tincture of digitalis of unknown relative strengths. The numbers indicate the order of their administration.

† "K" is "Q-T" interval divided by square root of cycle length; see text.

‡ Marked nausea.

be measured). The cycle length must, therefore, be taken into account. Although there is difference of opinion as to the formula best expressing the relationship, that used by Bazett (15) seems to fit well for usual heart rates, say 50 to 120, and we have employed it in this clinic. According to it the "Q-T" interval should equal the square root of the cycle multiplied by a constant ( $Q-T = K\sqrt{C}$ ). Variations in "Q-T" can then be expressed as variation in "K."<sup>1</sup>

A study of the values of "K" led us to believe we could do best by relying principally upon it, as the results seem fairly consistent. The number of observations is very small, but it was not our intention to apply the statistical method in this study. Only the results immediately before the first dose and after a standard interval are presented. The intermediate values were neither more discordant nor helpful.

In the first series (Tables II and III) three strengths of the same tincture of digitalis, "A," "B," and "C," were given, known to differ by as much as 25 per cent. In three of the four cases

<sup>1</sup>In this investigation we are not concerned with the question of the normal value of "K" which is in the neighborhood of 0.38. On the whole matter, see the reports of Cheer and his co-workers (16, 17).

in which all three tinctures were given sample "B" clearly had the greatest effect, and similarly in three of the four cases "C" had a greater effect than "A," as shown by the values of "K." The incomplete cases (5 to 8) happen to fit in with this conclusion. On the basis of the values of "K" these samples were put in the order  $B > C > A$ . This conclusion was confirmed by data supplied by the Department of Pharmacology, according to which the relative strengths of

TABLE III

Constant "K" before and after giving digitalis.  
Data from Table I

Sub- ject	Sample A			Sample B			Sample C		
	Before	After	Dif- ference	Before	After	Dif- ference	Before	After	Dif- ference
1	0.38	0.35	0.03	0.38	0.32	0.06	0.38	0.35	0.03
2	0.42	0.41	0.01	0.41	0.36	0.05	0.40	0.46	0.04
3	0.40	0.39	0.01	0.42	0.34	0.08	0.41	0.36	0.05
4	0.38	0.37	0.01	0.40	0.37	0.03	0.39	0.36	0.03
Sum			0.06			0.22			0.15
5				0.40	0.37	0.03	0.40	0.39	0.01
6	0.37	0.35	0.02						
7				0.41	0.33	0.08			
8							0.41	0.35	0.06

TABLE IV

Electrocardiographic measurements and constant "K" immediately before and 16 hours after giving samples of digitalis of unknown potencies\*

Sub- ject	Digitalis tincture†	P-R interval		T wave		Cycle length		Q-T interval		Constant "K"	
		Before	After	Before	After	Before	After	Before	After	Before	After
3 AF	A (2)									0.389	0.376
	B (1)	seconds ?	seconds ?	mm. 2.2	mm. 1.2	seconds 0.855	seconds 0.890	seconds 0.360	seconds 0.340	0.383	0.335
9 Rh CVD	A (1)	0.155	0.160	-1.0	+0.7	0.905	1.130	0.430	0.390	0.452	0.367
	B (2)	0.155	0.160	-1.0	+1.0	1.060	1.090	0.445	0.385	0.432	0.369
10 Rh CVD	A (1)	0.150	0.145	3.5	4.0	0.730	1.070	0.350	0.400	0.410	0.387
	B (2)	0.140	0.150	3.7	5.5	0.660	0.990	0.320	0.350	0.394	0.352
11 Normal heart	A (2)	0.150	0.145	3.2	3.7	0.760	0.910	0.355	0.380	0.407	0.398
	B (1)	0.155	0.160	1.5	1.6	0.620	0.855	0.320	0.358	0.406	0.387
12 Normal heart	A (1)	0.165	0.190	2.5	3.0	0.725	0.920	0.358	0.390	0.420	0.407
	B (2)	0.160	0.175	3.5	3.6	0.840	1.030	0.360	0.390	0.393	0.391
13 Rh CVD	A (1)	0.160	0.160	3.5	4.0	0.850	1.000	0.360	0.390	0.390	0.390
	B (2)	0.150	0.160	3.0	4.5	0.760	1.000	0.345	0.380	0.396	0.390

\* See notes to Table I.

† "A" and "B" are samples of standardized aged and fresh tinctures of digitalis (not known apart before the conclusions were reached). Numbers denote the order of administration.

the samples were  $B = 125$  per cent,  $C = 100$  per cent, and  $A = 75$  per cent.

The second series (Tables IV and V) included two tinctures, A and B, one known to be old and the other fresh. By misunderstanding, they were both given to us in weak form (4.5 per cent), instead of the usual concentration of 10 per cent.

TABLE V

*Constant "K" before and after giving digitalis.  
Data from Table III*

Subject	Tincture A			Tincture B		
	Before	After	Difference	Before	After	Difference
3	0.39	0.38	0.01	0.38	0.34	0.04
9	0.45	0.37	0.08	0.43	0.37	0.06
10	0.41	0.39	0.02	0.39	0.35	0.04
11	0.41	0.40	0.01	0.41	0.39	0.02
12	0.42	0.41	0.01	0.39	0.38	0.01
13	0.39	0.39	0.00	0.40	0.38	0.02
Sum			0.13			0.19

The effects were, therefore, not marked. Nevertheless, we concluded that "B" was somewhat stronger than "A," for in four of the six cases the changes in the value of "K" were in that direction, though the difference did not seem to be great. Data supplied after this decision was reached showed that standardization of the tinctures just before the first tests gave these results (the differences are not statistically significant):

Sample	Potency		
	Dog	Cat	Frog
A (Aged).....	0.90	0.95	0.84
B (Fresh).....	1.00	1.00	1.00

The same method was applied to the study of a third pair of unknown samples, the nature of which together with the results of the work is described elsewhere (18).

#### DISCUSSION

In the first series, the method employed enabled us to distinguish correctly between three samples of the same preparation of digitalis, which differed in strength by 25 per cent. The results do not lead us to believe we can confidently and

reliably distinguish between preparations differing by significantly smaller strengths. It is recognized that they need confirmation. The statistical method applied to a large series of cases might clarify the situation.

It should be noted that we are not prepared to say that the strengths of the samples are in direct proportion to the changes produced in the value of "K." There are some indications that the formula used may not apply to very slow or very fast rates. In slow rates a constant factor may be needed to express a natural limit of relative increase in "Q-T." Such a constant factor would then be taken into consideration in comparing proportionately changes in the value of "K."

On the basis of these results the common practice of giving adult patients of usual size a total dose of digitalis of 1.0 to 1.2 gram in terms of potent powdered leaves, seems justified. In other words, calculation of the dose is not in general necessary. In very small or in very large individuals, factors with which this investigation did not deal may modify the dose. There are suggestions that in advanced age susceptibility to digitalis is increased, but the question has apparently not been seriously studied. The maintenance dose does not seem to depend upon weight in any case (19), but rather on the amount in the body (20).

Standardization of digitalis for clinical use is necessary in order to establish the fact that a preparation is not inert, unduly feeble, or too strong.

As to the second series, the differences found seem to be slight. They were in the same direction as those shown by animal assays, even though these were not statistically significant. It is possible that the fresh tincture was a little more potent than the aged preparation. Our chief interest lies in the fact that there was no discrepancy between the animal and clinical standardizations.

It is evident that the greatest care is necessary in attempting to carry out the clinical standardization of digitalis. The only methods so far suggested which seem to offer the promise of reliable results are those of Gilchrist and Lyon, in which the effect of a sample is judged in patients the subject of auricular fibrillation by the drop in



ventricular rate in terms of the initial ventricular rate, with statistical control; and the method here reported of using the decrease in the "Q-T" interval in relation to the cycle length ("T-T" interval, preferably). For the latter method the use of a comparator, which has not been available to us, seems desirable. Careful study of the "P-R" interval and "T" wave should be made, as it is still uncertain to what extent these phenomena may be important aids in arriving at final conclusions. Those portions of the cardiac cycle which are used for correlation (such as cycle length and "Q-T" interval) should be measured in the same cycles. In both cases it is necessary to test the capacity of the method by giving different strengths of the same preparation to the same subject. It is clearly desirable likewise to give the various samples under trial to the same patients. For the second method, at present it seems desirable to use both normal subjects and patients with cardiovascular disease, but it is important to know to which group the subjects belong. The drug is best given in a single dose of a size determined from assay by a standard animal method, but well below the amount which might be expected to produce effects so marked as to mask differences between the samples being compared, at a definite time in relation to meals. The same dose of each of the samples to be compared should be given. It is believed that the following conditions should be met in making comparisons of the strengths of digitalis preparations:

Age limits, 20 to 50 years; careful study as to whether cardiovascular disease is present; no clinically demonstrable congestive failure; no fever for two weeks; no recent serious illness; no diarrhea or vomiting; no digitalis for 30 days; no important drug therapy (especially no opiate) during the tests; hospitalization and a constant diet throughout, or certainly during the tests; the observations to be compared should, if possible, be made at the same time of day, and under conditions as nearly comparable as they can be made.

#### SUMMARY

Past efforts to standardize digitalis by clinical study are briefly reviewed.

Attempt was made to ascertain the feasibility

of separating by clinical assay different strengths of the same tincture of digitalis. In the conditions of the experiments, among the phenomena observed (which included symptoms, weight, blood pressure, cardiac rate, and various aspects of the electrocardiogram), only one seemed clearly helpful. The effect, under standard conditions, of three such samples, on the "Q-T" interval of the electrocardiogram, stated in relation to the cardiac cycle length, made it possible to distinguish potencies related as 75:100:125. Aged and fresh samples of the same tincture, were similarly tested.

The conditions for satisfactory clinical assay of digitalis are discussed, and a standard set outlined.

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# A STUDY OF THE STANDARDIZATION OF DIGITALIS. II. THE RELATIONSHIP BETWEEN LABORATORY METHODS OF ASSAY AND POTENCY AS DETERMINED BY EXPERIMENTAL CUMULATIVE POISONING AND CLINICAL STANDARDIZATION

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It is commonly assumed that either frogs or cats may be used for the assay of digitalis, provided that suitable standards and experimental techniques are employed. Assay in frogs is the official method of the tenth revision of the United States Pharmacopoeia. The British Pharmacopoeia (1932) permits the use of both animals. Occasionally, however, there are encountered substances in digitalis leaf or glucosides from plants related pharmacologically to digitalis which are not of the same relative potency in frogs and in cats. The cumulation experiments in animals and the clinical experiments reported in this paper were undertaken to learn, in the case of two different samples of digitalis leaves, whether the results could be correlated with a method of assay.

## METHODS AND RESULTS OF ASSAY

Seven different samples of powdered leaves of *Digitalis purpurea* were systematically investigated to ascertain their toxicity toward frogs (*Rana nigromaculata*) and mammals (cats and dogs).<sup>1</sup> Extracts of each sample were made by using absolute alcohol in a Landsiedl extractor operated for eight hours (see Foster and van Dyke (1)). Two samples (D and F) were found to be equally toxic when administered to cats and dogs; in frogs, on the other hand, sample D was significantly more toxic than sample F. Tinctures freshly prepared were used to learn their cumulative potency in dogs and their effectiveness in the clinic.

In the preparation of the tinctures for injection into frogs, alcohol was removed by the method described previously (1). Reasonably accurate measurements of potency were made by using, in most cases, groups of twenty to thirty frogs.

Eighteen hours after the injection of the alcohol-free extract into the ventral lymph sac, the mortality rate was observed. It was usually necessary to estimate the dose which would have killed fifty per cent of a group of frogs; for this purpose we employed the table given in the Brit-

TABLE I  
The potency of leaf F relative to leaf D  
as determined by assay in frogs

Extraction number	Number of frogs used	Dose killing fifty per cent	Potency of F in terms of D*	Potency in terms of U.S.P. ouabain
		mgm. per kgm.		$\times 10^{-4}$
1 D	20	209	0.72	
1 F	20	289		
1 D	30	215	0.65	
1 F	30	333		
2 D	30	216	0.58	
2 F	30	370		
3 D	30	230	0.67	
3 F	30	345		
6 D	15	230	0.64	
6 F	15	357		
11 D	20	360	0.78	6.67
11 F	20	460		
Ouabain	20	0.240		5.22
11 D	20	310	0.70	6.55
11 F	20	445		
Ouabain	20	0.203		4.57
Internat. S.P.†	20	270		8.38
Ouabain	20	0.226		
Internat. S.P.	30	255		8.08
Ouabain	30	0.206		
Internat. S.P.	40	311		9.33
Ouabain	40	0.290		

\* Mean and standard error of mean potency of F in terms of D =  $0.68 \pm 0.024$ .

† Internat. S.P. = International standard of potency.

<sup>1</sup>We are indebted to Mr. F. A. Upsher Smith for samples of leaves of *Digitalis purpurea*.

ish Pharmacopoeia (1932). Preliminary experiments, which are not reported, were performed with batches of ten frogs each to learn the appropriate doses; this was necessary because of variations in susceptibility due to such factors as season and previous care. Complete assays of five different tinctures of each sample (D and F) were made. The mean and the standard error of the mean of all the measurements of the potency of leaf F in terms of leaf D were found to be  $0.68 \pm 0.024$  (Table I). The results were sufficiently consistent to justify the belief that leaf F

ration of meat, bread, and vegetables, as well as the housing of the dogs, was controlled. Ordinarily it was possible to inject only four dogs daily: one pair received suitable doses of tincture D and the other of tincture F. The tinctures were diluted with isotonic saline solution and thoroughly mixed just before intravenous injection (antibrachial, saphenous, or external jugular vein). No anesthetic was employed. It was not found feasible to make electrocardiograms routinely; the respiratory rate, heart rate, and peculiarities of cardiac rhythm, as well as the presence or ab-

TABLE II  
The potency of leaf F relative to leaf D measured in mammals

Group	Preparation	Animal	Number used	Mean and standard error of mean lethal dose	Potency		
					Animal	Of preparation	In terms of
1	Leaf D	Cat	10	mgm. per kgm. $70.2 \pm 4.71$	Cat	Leaf F	Leaf D: 1.04
2	Leaf D	Dog	9	$80.1 \pm 6.73$	Dog	Leaf F	Leaf D: 1.06
3	Leaf F	Cat	10	$67.7 \pm 5.41$	Cat	Leaf F	Internat. S.P.: 0.84
4	Leaf F	Dog	9	$75.6 \pm 3.30$	Cat	Leaf D	Internat. S.P.: 0.81
5	U.S.P. ouabain	Cat	10	$0.0807 \pm 0.0053$	Cat	Leaf F	Ouabain: 1.12*
6	U.S.P. ouabain	Dog	10	$0.0843 \pm 0.0062$	Cat	Leaf D	Ouabain: 1.05*
7	Internat. S.P.	Cat	7	$56.6 \pm 3.21$	Dog	Leaf F	Ouabain: 1.19*
					Dog	Leaf D	Ouabain: 1.15*

\*  $\times 10^{-3}$ .

had a potency about 70 per cent of that of leaf D in frogs.

All the mammalian (cats and dogs) assays were performed according to a modified Hatcher-technique similar to that of Wallace and van Dyke (2). The condition and previous care of the animals used, as well as the experiments themselves, were kept uniform. The assays were about equal (Table II). Leaf F in fact appeared to be slightly more potent, although the difference is not significant. The probability that the lethal doses for cats would be lower than those for dogs is 0.25 both for Groups 1 and 2 and Groups 3 and 4. The potencies of leaves D and F in terms of U. S. P. ouabain and international standard powder were approximately the same (Table II).

#### Cumulative poisoning in dogs

The cumulative effects of tinctures of samples D and F were assayed in dogs (2). Only healthy dogs, of comparable weight, were selected. The

sense of vomiting, salivation, and diarrhea, were however recorded daily before and one hour after injection.

In some of the hearts were found, at necropsy, several specimens of *Dirofilaria immitis*, but there was no evidence that the presence of these contributed to the death of animals. In six pairs no infection with filaria was present as ascertained by examination of blood smears and at necropsy (Table III, Groups 1 and 2). Seven pairs of animals, a fraction of which were filaria-infected, and six additional pairs, the group of filaria-free animals, comprise Groups 3 and 4 (Table III). No other abnormalities were found.

The animals were weighed once every 48 hours. There was progressive loss of weight averaging, at death, about 17 per cent.

If the mean survival periods of Groups 1 and 2 and 3 and 4 are compared (Table III), there is no evidence that leaf D differs significantly from leaf F. There were also no apparent differences

TABLE III

*Cumulative poisoning by tinctures of leaves D and F.  
Thirty-five per cent of a lethal dose was  
administered daily to each dog*

Group	Tincture made from	Animals				Mean and standard error of mean survival period	Remarks
		Number	Mean weight				
			At start	At death			
			kgm.	kgm.	Days		
1	Leaf D	6	13.1	11.0	$10.7 \pm 2.42$	No filaria infection	
2	Leaf F	6	12.8	10.7	$9.2 \pm 0.48$	No filaria infection	
3	Leaf D	13	12.9	10.7	$10.8 \pm 1.35$	Filaria infection in some animals	
4	Leaf F	13	12.9	10.7	$8.5 \pm 0.70$	Filaria infection in some animals	

in the effects of the two samples on the cardiac rate and rhythm. The probability that similar differences in the mean survival period would be encountered by chance is 0.56 for Groups 1 and 2, and 0.15 for Groups 3 and 4. It may therefore be concluded that the potencies of these tinctures (when used as cumulative poisons) were estimated more accurately by assay in cats and dogs than in

frogs. It is of interest, but of doubtful significance, that sample F, although much less potent in frogs, nevertheless appeared equal or slightly more potent in cats and dogs when assayed not only by acute experiment but also by the cumulation method.

### *Clinical measurements of the potency of the tinctures*

In a clinical assay it was possible to distinguish among three strengths of the same tincture, the relative potencies of which were 75:100:125. Were the frog method of assay to give values parallel with those found in the clinic, leaf F should turn out, in the clinic, to have only seventy per cent of the strength of leaf D—the relation actually found in frogs. In mammals, the two leaves were in fact equal.

For comparison with the assays in animals, the electrocardiograms of individuals, both normal and subject to cardiovascular diseases, were studied. All the subjects except 14 and 19 received courses of treatment with both tinctures

TABLE IV

*Electrocardiographic measurements and constant "K" before and after tinctures of leaves D and F*

Subject *	Digitalis ** tincture	"P-R" interval		T wave		Cycle length		"Q-T" interval		Constant "K" †	
		Before	After	Before	After	Before	After	Before	After	Before	After
3 AF	D (2)	seconds	seconds	mm.	mm.	seconds	seconds	seconds	seconds	0.369	0.347
	F (1)	?	?	3.0	2.5	0.730	1.080	0.315	0.360	0.362	0.316 ‡
12 Normal	D (1)	0.160	0.165	3.0	4.0	0.800	0.810	0.355	0.355	0.397	0.376
	F (2)	0.160	0.170	2.5	2.0	0.940	0.810	0.365	0.340	0.394	0.378
14 Normal	F (1)	0.145	0.160	3.0	2.5	0.665	0.705	0.315	0.305	0.386	0.363
	F (2)	0.150	0.160	3.0	2.5	0.640	0.950	0.310	0.340	0.388	0.349
15 Luetic CVD	D (2)	0.180	0.200	2.3	2.0	0.840	0.800	0.380	0.340	0.415	0.380
	F (1)	0.200	0.230	2.5	2.0	0.835	0.920	0.365	0.330	0.399	0.344
16 Con CVD	D (2)	0.165	0.200	1.3	1.3	0.610	0.605	0.330	0.320	0.422	0.411
	F (1)	0.165	0.210	1.5	0.5	0.590	0.560	0.320	0.280	0.417	0.374
17 Hy CVD	D (1)	0.140	0.150	0.3	1.0	1.140	1.160	0.400	0.370	0.375	0.344
	F (2)	0.130	0.160	1.0	0.5	1.080	1.135	0.400	0.380	0.385	0.333 ‡
18 ‡ Normal	D (2)	0.160	0.160	0.8	1.3	0.860	0.960	0.350	0.360	0.377	0.367
	F (1)	0.150	0.160	3.0	2.5	1.035	1.200	0.400	0.400	0.393	0.383
19 Normal	D	0.155	0.160	4.0	3.0	0.600	0.660	0.320	0.320	0.413	0.394

\* "AF" is auricular fibrillation; "CVD" is cardiovascular disease; "Con" is congenital; "Hy" is hypertensive.

\*\* The numbers in parentheses indicate the order of administration of the tinctures.

† "K" is "Q-T" interval divided by square root of cycle length.

‡ Marked nausea.

§ Only 0.8 proper dose of each tincture.

(Tables IV and V). Because of a misunderstanding Subject 3 was given 0.3 gram of digitalis leaf ten days before tincture F but in all probability without appreciably affecting the result. The average shortening of the constant "K" ( $= Q-T \text{ interval} / \sqrt{\text{cycle length}}$ ) was greater after taking tincture F, although it was consistently weaker by frog assay. The mean degree of shortening of "K" and the standard error of the mean were  $0.023 \pm 0.0056$  for tincture D and  $0.035 \pm 0.0099$  for tincture F. The difference between the means is not statistically significant. In dogs also tincture F appeared to be somewhat the stronger.

TABLE V

Constant "K" before and after tinctures of leaves D and F

Subject	Tincture D			Tincture F		
	Before	After	Difference	Before	After	Difference
3	0.37	0.35	0.02	0.34	0.32	0.02†
12	0.40	0.38	0.02	0.39	0.38	0.01
15	0.42	0.38	0.04	0.40	0.34	0.06
16	0.42	0.41	0.01	0.42	0.37	0.05
17	0.38	0.34	0.04	0.39	0.33	0.06†
18*	0.38	0.37	0.01	0.39	0.38	0.01
Sum			0.14			0.21
14				0.39	0.36	0.03
14				0.39	0.35	0.04
19	0.41	0.39	0.02			

\* Only 0.8 proper dose.

† Marked nausea.

#### DISCUSSION

The results of this investigation do not justify the generalization that assay of all or most samples of digitalis can be performed more usefully in cats or dogs than in frogs. More data with other samples are required. Our data offer support, however, for this generalization. Tinctures made of leaf F were significantly weaker by frog assay than those made of leaf D. On the other hand in the mammalian assays, in the clinical tests, and in the cumulation experiments in dogs, they were all equally potent. Tinctures of leaf F appeared in fact somewhat stronger than D-tinctures in mammalian experiments, although our data on this point are inconclusive.

A reason for the different results with F and

D may be due to a higher concentration of genins and a lower concentration of glucosides in leaf F. It appears to be the general conclusion (4, 6) that the genins are less potent (one-third to one-fifth) in frogs than the corresponding glucosides but have approximately equal strength in mammals (cats). Gröber (5) has come to a different conclusion, however; in frogs he thinks they (genins and glucosides) are equal, but in rabbits the genins about a third as powerful. It seems likely, furthermore, that genins are more loosely bound to cardiac muscle than are glucosides (7, 8) and also more easily excreted. The inference would be natural therefore that if the F-leaves were richer in genins they would be weaker clinically and in cumulation experiments than the D-leaves. But F-leaves appear on the contrary to be as potent as D-leaves; sometimes indeed more potent.

In the case of the glucoside scillonin, Wallace and van Dyke (2) found that slight cumulative poisoning was associated with a relatively low acute toxicity in frogs and a high acute toxicity in cats and dogs. These findings obviously are different from those sometimes encountered in similar experiments with digitalis tinctures.

#### SUMMARY

Two samples of leaf of *Digitalis purpurea* were found to be equally potent in mammals (cats and dogs) but to differ significantly in potency as measured by assay in frogs. They were also compared by means of cumulation experiments in dogs and by a satisfactory method of clinical assay.

The cumulation experiments in dogs (Table III) and the clinical assays (Tables IV and V) were in agreement with the assays in mammals (Table II).

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# CHANGES IN THE BLOOD AND CIRCULATION WITH CHANGES IN POSTURE. THE EFFECT OF EXERCISE AND VASODILATATION

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In the erect posture the forces tending to filter fluid from the blood into the tissues of the dependent parts are much greater than in the recumbent position. As Krogh, Landis and Turner have remarked, the erect human being is always close to edema (1). In a previous study of the effect of quiet standing (2) it was pointed out that the primary factors concerned in limiting the loss of fluid from the blood in the standing-still position appear to be an increasing concentration of plasma proteins, with a resulting rise in colloid osmotic pressure<sup>1</sup> and, probably, an increasing tissue pressure. However, secondary factors, changes in the circulation especially, modify this mechanism, particularly under conditions of normal activity. The changes may be the direct result of the change in posture, as for example the vasoconstriction which apparently accompanies the change in position. They may be the result of other influences such as exercise, or variations in environmental temperature. Under abnormal conditions, such as a nutritional hypoproteinemia, and particularly in border line states of such a disorder, variations in these factors might determine the occurrence or non-occurrence of edema. Therefore, we have studied the effect of some of these secondary factors on the circulation in the legs and on changes in the composition of the blood which occur in the erect position. The present paper deals with (1) the effect of muscular activity on the changes in the blood resulting from the erect posture, (2) changes in the circulation in the feet and legs in the erect posture, quiet and moving, as shown by changes in the surface temperature, (3) a comparison of the circulation time in the quiet and moving leg in the erect posture, and (4) the influence of vasodilatation

on the changes in the composition of the blood which occur on standing. For convenience in presentation, the methods and data in each of the studies will be presented separately.

## I. THE EFFECT OF MUSCULAR ACTIVITY ON THE CHANGES IN THE BLOOD RESULTING FROM THE ERECT POSTURE

The influence of muscular activity was determined by comparing the changes in the composition of the blood and in the leg volume of an actively moving leg in the erect posture with those of the opposite leg which was kept motionless. In addition a few observations were made of the capillaries in the great toe of the quiet leg.

The subjects consisted of six young healthy men (students) and one man with nutritional edema. About an hour after a light lunch the subject reclined for 60 minutes or more. During this time the capillaries at the base of the great toe nail were observed with a capillary microscope. At the end of the reclining period a sample of blood was drawn from the arm.<sup>2</sup> After the blood was drawn the venous pressure was measured directly. The volume of each leg was then determined by measuring the amount of water displaced from a rigid metal boot reaching to about the knee (48.3 cm.). The temperature of the water was adjusted to approximately room temperature. After the immersion the leg was quickly dried without rubbing. Each measurement occupied approximately five minutes and the subject reclined five minutes between the measurements of the first and second legs. After the volume of the second leg was measured the subject again reclined for a period of five to ten minutes. The subject then mounted a stationary bicycle, the left pedal of which was removed. The left leg was allowed to hang

<sup>1</sup> The increase in colloid osmotic pressure is greater than the increase in concentration of the proteins because of the increase in specific osmotic pressure, i.e., pressure per gram of protein.

<sup>2</sup> It is assumed that during reclining, blood from the foot has the same composition as blood from the arm (2).

perpendicularly, supported comfortably in half the experiments by a platform and unsupported in the others. The subject began to pedal immediately with the right leg against a resistance which was obtained by means of an adjustable spool pressing against the partially deflated tire of the rear wheel. The position assumed is believed to be comparable, as far as the left (motionless) leg is concerned, to standing at an angle of 75 degrees as employed in previous experiments (2). Pedalling was continued at a rate of forty to fifty revolutions per minute except for short interruptions to allow for the inspection of the capillaries in the toe of the motionless foot. When the latter was allowed to hang unsupported, it was supported temporarily while the capillaries were observed. When the right leg was not pedalling it was stopped at the mid point between the top and bottom of the stroke. Total pedalling time was thirty minutes but the time the subject was on the bicycle varied because of the time taken out for capillaroscopy and the drawing of samples of blood at the end of the period of pedalling. When the subject had pedalled for thirty minutes a sample of blood was drawn from each foot from a vein on the dorsum or at the internal malleolus. The sample from the right (moving) foot was always drawn first and the venous pressure was determined in the left foot. The volume of each leg was then measured again. Because of difficulties sometimes encountered, the time relation of the various procedures, particularly the time elapsing between the cessation of pedalling, the obtaining of the two samples of blood and the final measurement of leg volume, was not the same in all the experiments. The samples of blood were drawn under oil without stasis, and with heparin as an anticoagulant; specimens were removed for determining the concentration of plasma proteins, the colloid osmotic pressure and the nonprotein nitrogen. The methods of analysis were the same as those described in a previous paper (2).

The results of the experiments are summarized in Table I. In four experiments the increase in the concentration of total proteins both actual and percentile, was greater in the "motionless" (left) leg than in the "moving" (right) leg.<sup>3</sup> In two,

<sup>3</sup> This is believed not to be the result of a lessened tendency to filtration, resulting from the intermittent na-

the increase was practically the same in each leg, the differences being within the limit of error. Absence of a greater difference between the two legs in some of the experiments may have been the result of such irregularities as delay in drawing samples of blood and fainting of the subject. The changes in the concentration of albumin were similar, the one instance in which the increase in concentration of albumin was greater in the "moving" leg than in the "quiet" leg occurring in the experiment in which the increase in total protein was slightly greater in the moving leg. In two instances the concentration of globulin was greater in the moving than in the quiet leg. When the percentile increase of total protein and of the two fractions are compared discrepancies are found which suggest that variable amounts of either albumin, globulin or both were lost through the vessel wall. There is no evidence, however, of any consistency in respect to the amount or kind of protein which escaped. This lack of consistency is in accord with the observations of Landis and his associates (3).

In every experiment the increase in colloid osmotic pressure of the blood was greater in the "quiet" leg, but in Subject H the difference was very slight, almost within the limit of experimental error. The increase in leg volume was greater in the "quiet" leg than in the "moving" leg in all the experiments. In two experiments, the right or "moving" leg not only failed to show as great an increase in volume as the left but showed an actual decrease compared with its volume during the reclining period. In one of these two experiments the subject was the patient with edema. Although the method of measuring leg volume is not very accurate, the limit of error, which is believed to be within ten or fifteen cc., is much less than the changes in volume of the two legs or than the differences in change of volume between the two legs, even in those experiments in which a decrease in volume of the "moving" leg was found.

No study was made of the quantitative changes in the capillaries in the left (quiet) foot but the

ture of the venous pressure in the moving leg, the somewhat smaller hydrostatic pressure during part of the cycle of movement, or the effect on leg volume of a possibly lessened filling of the blood vessels in the moving leg.

TABLE I

*The concentration of plasma proteins, colloid osmotic pressure, venous pressure, and volume of the leg in the reclining position and in the quiet and moving legs in the erect posture*

Subject	Position †	Serum proteins						Osmotic pressure		Leg volume		Ve-nous pres-sure	Remarks
		Total		Albumin		Globulin							
			In-crease		In-crease		In-crease		In-crease		In-crease		
B. D.	Reclining	grams per cent		grams per cent		grams per cent		cm. H <sub>2</sub> O		cc. H <sub>2</sub> O		cm. H <sub>2</sub> O	Delay in drawing blood samples from feet Fainted
	Erect R L	6.83		4.75		2.08		34.4		R 3580 L 3490		11.7	
		7.64 7.88	11.9 15.4	5.32 5.36	12.0 12.8	2.32 2.52	11.5 21.1	40.5† 42.0†	17.8 22.1	3595 3590	15 100	112.8	
S. B.	Reclining	6.65		4.76		1.89		35.3		R 3430 L 3490		8.3	Fainted
	Erect R L	7.24 7.31	8.9 9.9	5.22 5.36	9.7 12.6	2.02 1.95	6.8 3.2	43.3 44.0	22.7 24.6	3433 3565	3 75	111.5	Vomited
		Reclining	6.84		4.49		2.35		34.7		R 5420 L 5175		7.1
J. D.	Erect R L	7.86 9.21	14.9 34.6	5.15 6.08	14.7 35.4	2.71 3.13	15.3 33.2	42.5 55.4	21.6 59.7	5310 5240	-110* 65	116.1	
		Reclining	6.50		4.83		1.67		34.2		R 3572 L 3712		5.4
	H. G.	Erect R L	7.43 8.16	14.3 25.5	5.46 5.90	13.0 22.2	1.97 2.26	17.9 35.3	42.4 47.0	24.0 37.4	3630 3822	58 110	111.0
Reclining			6.70		4.85		1.85		36.1		R 4140 L 4050		10.0
R. H.		Erect R L	7.47 7.42	11.5 10.7	5.44 5.33	12.2 9.9	2.03 2.09	9.7 13.0	41.3 42.5	14.4 17.7	4220 4200	80 150	109.4
	Reclining		6.20		4.11		2.09		29.5		R 4360 L 4410		5.4
	F. I.	Erect R L	7.14 7.35	15.1 18.5	4.63 5.00	12.7 21.4	2.51 2.35	20.1 12.4	36.5 38.8	23.7 31.5	4310 4570	-50* 160	125.7
Reclining			6.20		4.11		2.09		29.5		R 4360 L 4410		5.4

\* Decrease.

† R = Right leg; L = Left leg.

‡ Calculated osmotic pressure using formula of Wells, Youmans and Miller (J. Clin. Invest., 1933, 12, 1103).

following qualitative changes were observed. During the reclining period the observable capillaries were usually rather few in number; the arterial and venous limbs were of about the same diameter, rather narrow and light red in color. The blood flow through them was relatively rapid. With the subject on the bicycle the number of visible capillaries rapidly increased. At the same time the diameter of the loops increased, that of the venous more than the arterial, with the former often appearing engorged with blood. The color of the blood became much darker and the flow slower. These changes increased as time elapsed. In one subject (H. G.) the arterial limbs became greatly constricted toward the end of the experiment, and in another (R. H.) it was noted that

when venipuncture was performed in that leg the capillaries "collapsed" (became constricted?). The subject with edema had fewer visible capillaries in the reclining position than the normal subjects, and when he mounted the bicycle, though more capillaries became visible, the cyanosis was less and the increase in the size of the venous loop over that of the arterial was less.

### Summary

In the erect posture active muscular movements of the leg are accompanied by less concentration and less rise in colloid osmotic pressure of the blood in the moving leg than in the other leg kept motionless. At the same time there is a smaller

increase in the volume of the moving leg, and, in some cases, an actual decrease compared with its volume in the reclining position. In the quiet leg in the erect posture the capillaries of the toe are dilated and the number of open capillaries increased.

II. CHANGES IN THE CIRCULATION IN THE FEET AND LEGS, QUIET AND ACTIVE, AS SHOWN BY CHANGES IN THE SURFACE TEMPERATURE

Changes in the circulation in the feet and legs under different conditions of posture, exercise and temperature were studied by observing the changes in the surface temperature.

The subjects were young, healthy, men and women. The following general procedure was followed with certain variations which will be described below. The subjects reclined with the feet and legs exposed to above the knees for an hour or so. During this period the surface temperature of the feet and legs, at the base of the great toe, over the external malleolus and over the mid leg on the anterior surface, was recorded with a thermocouple.<sup>4</sup> Room temperature, which was maintained as constant as possible, with a variation of about one degree centigrade, was measured with a mercury thermometer suspended near the feet and legs. After the surface temperature of the feet and legs had become relatively constant the subject stood as quietly erect as possible for periods of 60 to 75 minutes, and the surface temperatures were recorded at frequent intervals. Contact of the feet with the floor was prevented by a blanket. After the standing period the subjects again reclined, and the changes in surface temperature were followed. In most of the experiments the environmental temperatures were

rather high, 28 to 30° C. Some of the experiments were performed in a constant temperature room at temperatures of 19 to 24° C.

In order to compare the effect of exercise and quiet standing on the surface temperature of the feet and legs, the following experiment was performed. After the initial period of reclining the subject mounted a stationary bicycle so arranged that he was able to stand nearly erect and quietly on the left leg while pedalling against an adjustable resistance with the right. Pedalling was continued for approximately one hour, except for brief periods to determine the surface temperatures which were measured at intervals of about five minutes in each leg. At the end of the period on the bicycle the subject again reclined, and the changes in the surface temperature were followed further.

The results are summarized in Tables II and III, and typical examples are illustrated in the accompanying charts. In nearly every instance the assumption of the standing-still posture was followed by a drop in the surface temperature of the toe (Table II) (Figure 1). As a rule, the

TABLE II  
*Changes in the surface temperature of the feet (toe) during quiet standing*

Subject	Room temperature	Temperature before standing	Lowest temperature while erect	Maximum decrease	Time to reach lowest temperature	Temperature at end of standing	Standing time
	° C.	° C.	° C.	° C.	minutes	° C.	minutes
H. G.	28.8 to 29.3	34.0	32.0	2.0	70	32.8	75
E. W.*	28.7 to 29.3	31.9				32.6	61
A. D.	28.8 to 29.3	32.5	31.6	0.9	47	32.2	71
V. S.	28.7 to 29.3	32.0	30.1	1.9	21	30.8	73
P. D.	28.8 to 29.2	32.3	29.6	2.7	61	30.0	76
V. S.	22.0 to 24.0	26.1	25.1	1.0	20	25.1	40
A. D.	23.5 to 24.5	27.7	26.2	1.5	33	27.1	38
P. D.	24.8 to 25.5	29.8	28.7	1.1	32**	28.7	33
J. Y.	19.0 to 22.0	28.8	24.6	4.2	60	24.6	60
H. F.	19.5 to 21.0	28.8	24.9	3.9	40	25.2	60
M. C.	19.5 to 21.0	29.3	27.0	2.3	20	29.8	60

\* Surface temperature rose while standing.  
\*\* Fainted twice.

<sup>4</sup> The instrument used was a Tycos "Dermatherm." Freeman and Linder (Arch. Int. Med., 1934, 54, 981) have recently pointed out the relatively large errors which may occur in measurements of surface temperature with a thermocouple. We are cognizant of these possible errors and have controlled them as far as possible. Care was taken that exactly the same spot was tested each time. Although equilibrium of the skin with the surrounding air may not always have been reached during the preliminary period, in view of the consistency and magnitude of the changes the results are believed to be significant.

drop began very soon after the erect posture was assumed. In some experiments the surface temperature continued to fall uninterruptedly until a minimum was reached. In others the decline was irregular, being interrupted by periods during which the temperature remained constant or even

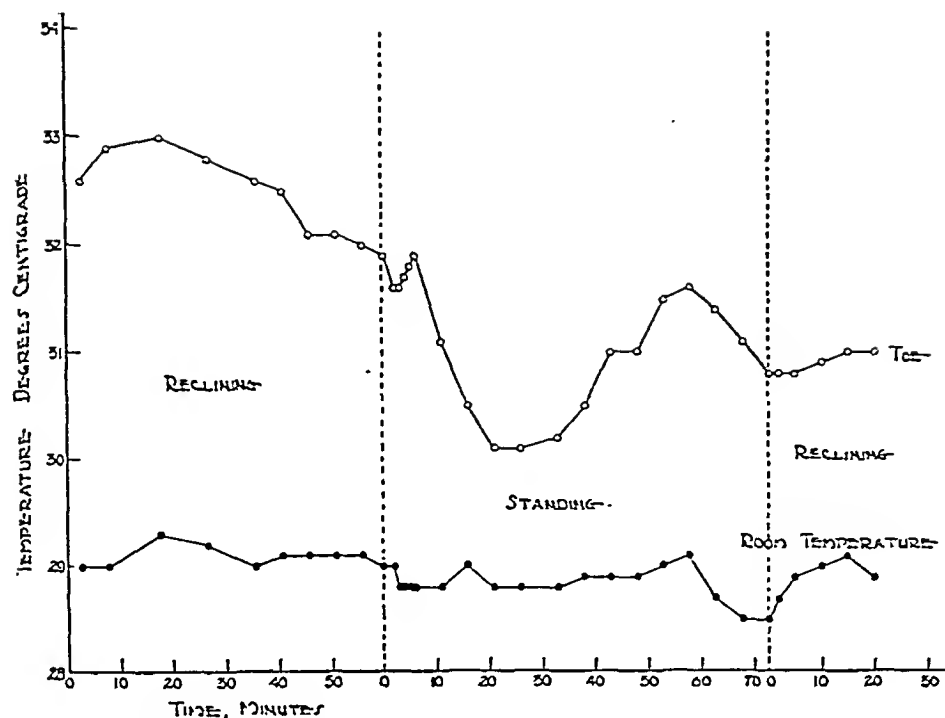


FIG. 1. CHANGES IN THE SURFACE TEMPERATURE OF THE FOOT (TOE) DURING QUIET STANDING

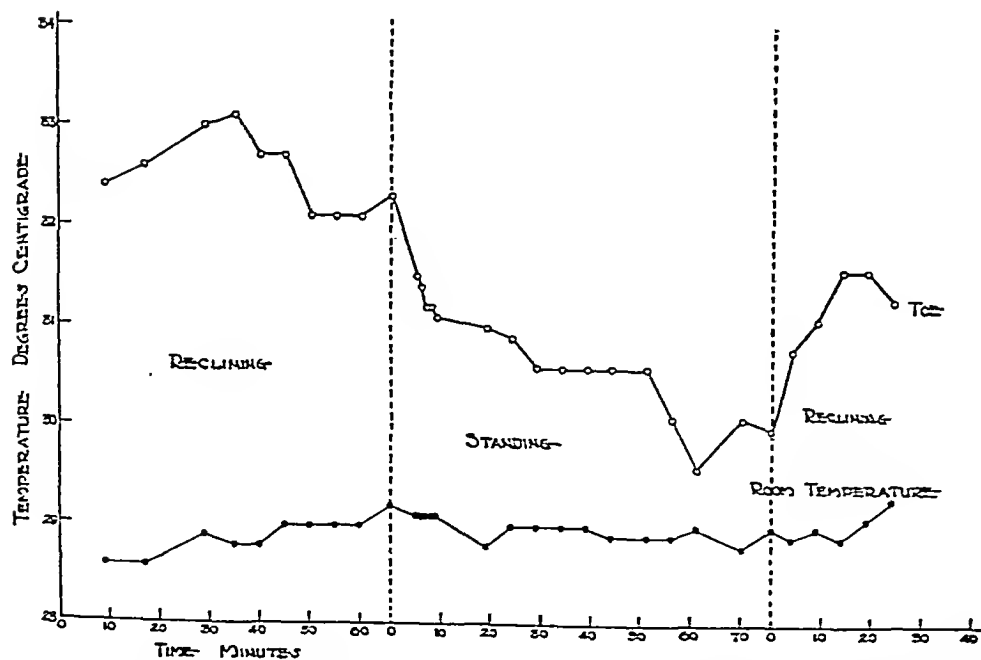


FIG. 2. CHANGES IN THE SURFACE TEMPERATURE OF THE FOOT (TOE) DURING QUIET STANDING

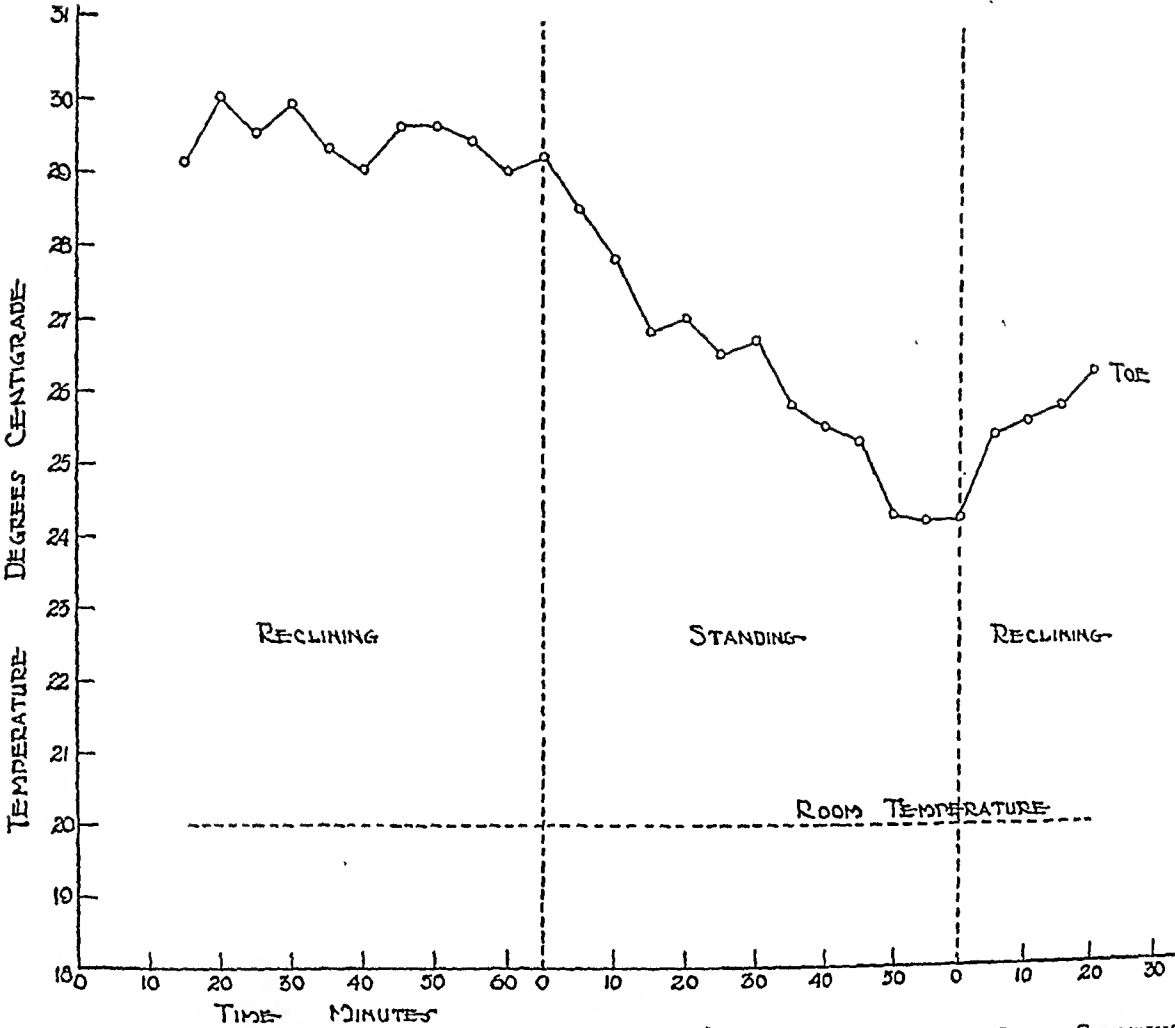


FIG. 3. CHANGES IN THE SURFACE TEMPERATURE OF THE FOOT (TOE) DURING QUIET STANDING IN A COOL ENVIRONMENT

In this chart the surface temperatures have been adjusted to a room temperature of 20° C. by the use of Vincent's factor (Vincent, J., *La Temperature Climatologique, Ceil et Terre*, 2d series, 1890, 5, 515).

rose more or less for a variable period (Figure 2). The maximum fall in temperature varied from 0.9 to 4.2° C., and the time to reach the lowest point ranged from 20 to 70 minutes after the standing position was assumed. In one subject no fall occurred on standing, the temperature rising steadily for 26 minutes from the time she stood, following which it slowly fell until, after 61 minutes of standing, it had returned nearly to its level at the beginning of the standing period. In this experiment, however, the surface temperature of the toe had dropped rather abruptly just before the subject stood. In several experiments it was noted that following the maximum fall there was a greater or less return of the surface temperature toward the initial level (Figure 1). The surface temperatures of the ankle and leg, which are not included in the charts, showed

changes similar to those of the toe but of a smaller magnitude. No special difference was noted when

TABLE III  
*Changes in the surface temperature of the feet (toe) during the erect posture; comparison of the quiet and moving leg*

Subject	Room temperature	Temperature before standing	Lowest temperature while erect	Maximum decrease	Time to reach lowest temperature	Temperature at end of standing
	° C.	° C.	° C.	° C.	minutes	° C.
J. Y.	24.6 to 25.1	33.4	28.8	4.6	65	28.8
Quiet leg		32.9	28.4	4.5	65	28.4
Moving leg	26.0 to 26.5	32.1	29.6	2.5	50	29.7
J. A.		32.2	29.6	2.6	40	29.8
Quiet leg	24.9 to 25.6	29.1	28.5	0.6	5	28.3
H. F.		30.2	28.7	1.5	30	29.5
Quiet leg	25.2 to 26.2	31.4	29.8	1.6	25	31.4
M. C.		32.3	28.8	3.5	50	29.3
Quiet leg						
Moving leg						

the environmental temperature was lower, except that a lower level of surface temperature was reached than in the warmer environment (Figure 3). In most of the experiments resumption of the reclining position after standing was associated with a return of the surface temperature toward the prestanding level.

When the subject stood quietly on one leg and pedalled with the other, there was a drop in the surface temperature of both feet (Table III) (Figure 4). The fall was sometimes greater in the moving leg, and this leg less often showed a return of the surface temperature toward the ini-

tial level as the erect position was maintained. As in the other experiments the quiet leg became cyanotic but the moving leg was lighter in color and pink. The finding of a decrease in the surface temperature of the moving leg was somewhat surprising, as there is every reason to believe that the total blood flow through the moving leg is not only greater but faster than in the quiet leg. In part, the drop in surface temperature may have been due to the cooling effect of movement in the air. The appearance of the leg, however, suggested that in addition to any cooling effect from motion there was an actual decrease in blood flow

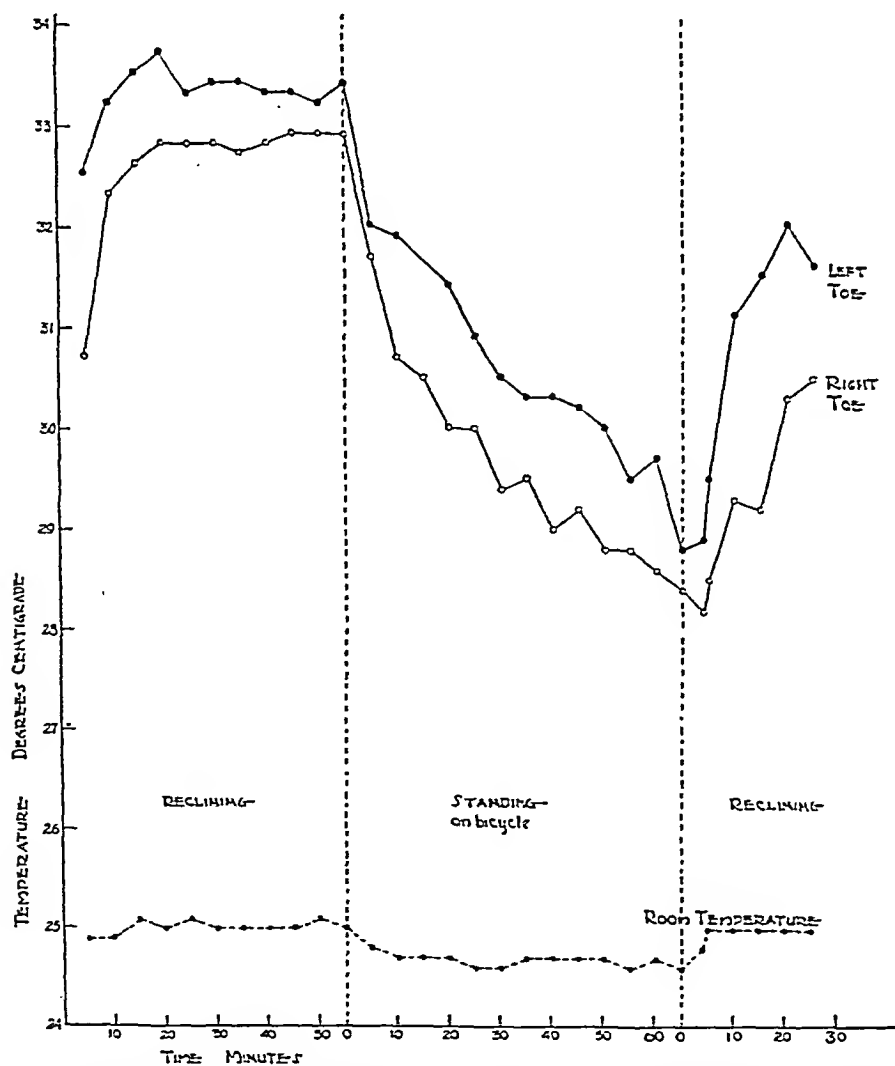


FIG. 4. COMPARISON OF THE CHANGES IN THE SURFACE TEMPERATURE OF THE FOOT (TOE) IN THE QUIET (LEFT) AND MOVING (RIGHT) LEG IN THE ERECT POSTURE



in the skin and subcutaneous tissues in contrast to the presumably much greater flow through the muscles. Therefore, changes in surface temperature are probably a poor index of total blood flow in the actively moving foot and leg.

As an incidental finding in these and certain other related experiments it was observed that venipuncture in either arm or leg was followed by a prompt and significant fall in the surface temperature of the feet (and usually of the legs). The decrease amounted to as much as  $2^{\circ}$  and persisted for as long as 15 to 20 minutes. False venipuncture, i.e. piercing the skin with the needle without entering the vein, usually caused a much less or insignificant effect. The drop in surface temperature of the legs and feet following venipuncture is apparently a reflex phenomenon similar to that caused by other physical and mental stimuli. Venipuncture appears, however, to be a more powerful stimulus to vasoconstriction than, for example, a similar degree of pain unaccompanied by injury to a vein, and is a factor for which allowance should be made when venipunctures are performed in conjunction with measurements of surface temperature.

### *Summary*

Assumption of the erect posture is usually accompanied by a prompt and significant fall in the surface temperature of the feet and legs. This drop occurs in a warm as well as in a cool environment, and in the muscularly active (pedalling) leg as well as in the quiet leg in spite of a presumably greater and more rapid total blood flow in the former.

### III. CIRCULATION TIME IN THE QUIET AND MOVING LEG IN THE ERECT POSTURE

The circulation time in the leg in the recumbent and erect positions and the differences in the quiet and moving leg in the latter position were determined as follows. The subjects were three healthy young men. An attempt to include two female subjects failed because of difficulty in performing venipunctures in the foot and ankle of the "moving" leg and the distress caused by the experiment. The subject reclined with the feet and legs exposed to above the knees for an hour or more. After this preliminary period the

circulation time in an arm and each leg was measured. The subject then mounted a bicycle so arranged that he stood nearly upright on the left leg while pedalling with the right, keeping the left practically quiet in spite of the action of the other leg. Pedalling against resistance was continued at the rate of approximately 50 cycles per minute for periods of 49 to 69 minutes, except for brief interruptions to inject the test solutions. An attempt was made to measure the circulation time in each leg at the beginning, middle and latter part of each period of standing and in the arm at the end of this period, but was completely successful in only one experiment. In two experiments the circulation time was determined by the injection of sodium cyanide (4) and in one experiment by the injection of either sodium dehydrocholate (5) or saccharin (6). Stop watches were used by two observers to register the time of the "reaction," the average being taken. Unless a definite reaction was obtained a second test was made a few minutes later. During the experiment the surface temperatures of the feet and legs were measured at frequent intervals, but the complicating effects of venipuncture and reactions to the intravenous injections obscure the relation of the changes in surface temperature to posture and exercise.

All the subjects found the procedure a very trying ordeal. When sodium cyanide was used to measure the circulation time in the legs, each injection was accompanied by a period of faintness, dizziness and, in some instances, nausea. This was particularly severe in the case of the left leg (quiet standing position) and may have been related to the large doses which were necessary in these circumstances. As Bock, Dill and Edwards have pointed out (7), with the great slowing of the circulation it is necessary to employ stronger concentrations of the drugs to secure a sharp end point. In one subject, not included in this series, a slough followed the injection of a small amount of cyanide solution (2 per cent) into the tissues of the foot outside the vein, with the formation of an ulcer which was very slow to heal. The injection of saccharin caused severe pain along the course of the vein in the leg, followed by faintness and later by a venous thrombosis. Subjects P. D. and A. D. nearly fainted toward the end of the experiment. These com-

TABLE IV

*Circulation time in arm\* and each leg\*\* while reclining and while erect with left leg motionless and right leg moving*

Posture	Circulation time		
	Subject H. G.	Subject P. D.	Subject A. D.
	<i>seconds</i>	<i>seconds</i>	<i>seconds</i>
Reclining			
Arm.....	34.6	14.6	21.0
Left leg.....	41.1	40.0	40.4
Right leg.....	49.0	39.2	40.9
Erect			
Left leg.....	144.0 (After 18 minutes)	150.0 (After 13 minutes)	76.6 (After 17 minutes)
Right leg.....	37.0 (After 20 minutes)	52.3 (After 23 minutes)	57.9 (After 36 minutes)
Left leg.....	77.4 (After 31 minutes)	62.0 (After 32 minutes)	146.2 (After 44 minutes)
Right leg.....	31.8 (After 40 minutes)	25.5 (After 44 minutes)	
Left leg.....	71.0 (After 49 minutes)		
Right leg.....	33.9 (After 57 minutes)		
Arm.....	10.0 (After 65 minutes)		27.0 (After 62 minutes)

\* Arm to carotid.

\*\* Leg to carotid.

plications may have caused some irregularities in the results.

The results are summarized in Table IV. In all three subjects the circulation in the left (quiet) leg was much slower in the erect than in the reclining position, the maximum slowing occurring in Subject H. G., in whom the circulation time was almost four times as long 13 minutes after the standing period began as it had been while reclining. In all three subjects the circulation while erect was much slower in the left (quiet) leg than in the right (moving) leg. The latter showed, in general, a faster circulation while erect and moving than when reclining and quiet. In two determinations the circulation time in the right moving leg was slightly longer when erect than when reclining, but it was necessary to stop the movement of the leg while the solution was injected, and some delay in this maneuver may have allowed the circulation to slow somewhat. In two of the subjects the circulation in the left (quiet) leg was much slower in the early than in the later part of the standing period. In Subject A. D., the circulation time in the quiet left leg was longer in the later part of the standing period than near the beginning. At this time, however, he was faint and suffering considerable pain.

### Summary

In the erect posture the circulation time in the quiet leg is much longer than in the reclining position. On the contrary, the circulation time in the moving leg is often shorter than in the reclining position, and hence often several times shorter than in the opposite, quiet leg.

#### IV. THE INFLUENCE OF VASODILATATION ON THE CHANGES IN COMPOSITION OF THE BLOOD WHICH OCCUR ON STANDING

In order to study the part played by vasoconstriction in the changes in the blood which accompany the erect posture, we abolished or lessened it, and compared the ensuing changes in the blood in the feet and legs during standing with those which occurred when vasoconstriction was present.

Various methods for obtaining a vasodilatation in the feet and legs were considered, but for several reasons the one which seemed most suitable and was adopted was that of heating the hands and arms (8). This procedure will produce in most normal subjects a considerable degree of vasodilatation in the feet and legs, which can be measured by determining the surface temperature.

The complete study consisted of four distinct experiments, as follows: First, a control experi-

ment to determine the maximum dilatation (increase in surface temperature) in the feet and legs, produced by heating the arms with the subject reclining. Second, a control experiment with the subject standing quietly, to determine whether heating of the arms could overcome the drop in surface temperature (decreased blood flow—vasoconstriction?), which we have shown occurs in the erect posture. Third, a determination of the degree of concentration of the blood (foot) and the change in leg volume, which occurred when the subject stood for a given period *without* vasodilatation. Fourth, a determination of the degree of concentration of the blood (foot) and the change in leg volume when the subject stood for a similar period *with* vasodilatation.

Two young healthy men served as subjects. The individual experiments were performed on separate days at varying intervals. In all the experiments the subjects first reclined with the feet and legs exposed to above the knees. The surface temperatures at the base of the great toe, over the external malleolus and just below the knee were recorded with a thermocouple, exactly the same location being tested each time. The room temperature was maintained as constant as possible in the neighborhood of 20° C., with a range of 2° C., and was recorded by a mercury thermometer suspended near the feet and legs. The hands and arms were heated by being immersed to above the elbows in water at a temperature of 44° C. or more. White enamel basins with covers were used, and the temperature was maintained by the addition of hot water at suitable intervals. Samples of blood were drawn without stasis, and those for serum protein and colloid osmotic pressure determinations under oil. The relative cell volume (hematocrit) was measured with Wintrobe hematocrit tubes (9) using heparin as an anticoagulant. The volume of the leg was measured as previously described.

In the first control experiment the subjects reclined until the surface temperatures were relatively constant. The hands and arms were then immersed in the hot water. Both subjects showed an increase in the surface temperature of the toes from a "pre-heating" level of 26.1 and 26.9 to 30.5 and 30.0° C., an increase of 4.4 and 3.1° C., respectively. The increase began 11 and 7 min-

utes after the hands and forearms were immersed, and the maximum was reached in 37 and 41 minutes. In both subjects the period before the temperature began to rise was marked by an initial drop. An initial drop in the surface temperature of the toe when the hands and forearms were first immersed in hot water, amounting to as much as 2° C. and persisting for as long as 11 minutes, often occurred in these experiments. In neither subject did the surface temperature of the toe reach 32° C., which is considered to indicate complete vasodilatation (Landis). This is probably explained by the fact that the temperature at the base of the toe was measured instead of at the base of the toe nail, where there is a richer anastomosing circulation. The ankle and leg showed a slight rise only.

In the second control experiment, after the preliminary reclining period, Subject W. H. stood and immediately immersed his hands and forearms in the hot water. Contact of the feet with the floor was prevented by a blanket. Quiet standing was maintained for a period of 41 minutes, during which time the surface temperature of the toe rose from the prestanding level of 24.8 to a maximum of 30.6° C. an increase of 5.8 degrees, 27 minutes after standing began. The rise started 11 minutes after standing and heating the hands and forearms began, the preceding interval being marked by a rather sharp drop of 1.5° C. To lessen this initial drop, the forearms and hands of the second subject (J. P.) were placed in hot water for 38 minutes before he stood. He then stood, keeping the forearms in hot water constantly for 36 minutes. While he still reclined with the arms in hot water, the surface temperature of the toes rose from 27.1° C. to a maximum of 29.9 degrees, 30 minutes after heating the forearms began. On standing, a drop of 1.1° C. occurred over a period of 9 minutes. The temperature then rose to a maximum of 30.9° C., 19 minutes after he stood, an increase of 3.8 degrees over the preheating period. Thus, heating the forearms and hands was able not only to overcome the drop in surface temperature which occurs on standing but was capable of increasing the surface temperature significantly above the preliminary reclining period. In this second experiment, in which the subject stood, the surface

temperatures of the ankle and of the leg not only showed a smaller rise than that of the toe but often remained constant or even dropped slowly but steadily.

In the third and fourth experiments, the effect of standing on the concentration of blood in the feet and on the leg volume, with and without vasodilatation, were compared. The subjects reclined until the surface temperature of the exposed feet and legs was quite constant and the concentration of blood in the upper and lower parts of the body had become equalized. The venous pressure in the arm was then measured, a specimen of blood was removed for the various analyses, and the volume of the leg measured. The subject then stood quietly for a period of 45 minutes, during which the changes in surface temperature were recorded. At the end of the standing period the venous pressure at the ankle was measured, a second sample of blood was drawn and the volume of the leg measured again. In Experiment 3, the hands and forearms were not heated. In Experiment 4, W. H. immersed the hands and forearms in hot water immediately on standing. With Subject J. P. the forearms and hands were immersed for 25 minutes before he stood, as well as during the standing period.

In these experiments the complicating effect of venipuncture, which causes a drop in the surface temperature of the feet and legs, was introduced. Because of this the surface temperatures at the time of standing were lower than in the control experiments. With Subject W. H., the temperature of the toe at the time of standing without vasodilatation was  $22.0^{\circ}$  and during the standing period it fell gradually to  $20^{\circ}$  C. With vasodilatation the temperature at the beginning of standing was  $21.9^{\circ}$  but it dropped irregularly to  $21.2^{\circ}$  C. over a period of 18 minutes, before it rose to a maximum of  $23.2^{\circ}$  C., about 3 degrees higher than in the experiment without vasodilatation. However, he was able to stand with the arms in hot water for a period of 30 minutes only before syncope stopped the experiment, and thus for only about 10 minutes of the standing period was the temperature of the toe much higher than in the experiment without vasodilatation. With Subject J. P. the surface temperature of the toe while standing without vasodilatation ranged from  $21.5$

to  $22.2^{\circ}$ . On standing with the arms heated the surface temperature of the toe rose from about  $23^{\circ}$  to a maximum of  $28.2^{\circ}$  and during the entire standing period was considerably higher than without vasodilatation, the maximum difference being about  $7^{\circ}$  C. In both subjects the surface temperature of the leg fell slightly on standing in spite of heating of the arms. The ankle showed a little rise. When the arms and hands were heated a vasodilatation was shown by a pinkness of the feet which was in sharp contrast to the cyanosis which appeared in the absence of heating. This was less noticeable in Subject W. H. Both subjects sweated profusely during the vasodilatation and both found the experiment a very trying ordeal.

The changes in the composition of the blood were exactly opposite in the two subjects (Table V). Subject W. H. showed a slightly greater concentration of the blood on standing with vasodilatation than without, as shown by the concentration of total protein and albumin and the increase in colloid osmotic pressure and relative cell volume. The difference in total protein was so slight, however, as to be of little significance. Changes in the concentration of globulin were the reverse of those of albumin and total protein, probably the result of a difference in the loss of the two protein fractions through the capillary wall (2). In keeping with the greater concentration of the blood during vasodilatation the increase in leg volume was greater with vasodilatation than without. Venous pressure during standing was essentially the same with and without vasodilatation. Subject J. P., in contrast to Subject W. H., showed less concentration of the blood with vasodilatation. The difference was consistent and was reflected in the changes in total protein, albumin, globulin, colloid osmotic pressure and relative cell volume. There was, however, little difference in the increase in volume of the leg in the two experiments, though the slight difference noted was in keeping with the changes in the blood. The fact that there was nearly the same increase in leg volume, in spite of less concentration of the blood and excessive sweating, suggests that the total filtrate was greater with vasodilatation. As in the case of Subject W. H., there was no difference in venous pressure, with or without vasodilatation.

TABLE V

*The concentration of plasma proteins, colloid osmotic pressure, relative cell volume, venous pressure and volume of the leg in the reclining and standing still positions, with and without vasodilatation.*

Sub- ject	Experi- ment	Posi- tion	Plasma proteins									Osmotic pressure			Leg volume		Relative cell vol- ume (hematocrit)			Ve- nous pres- sure
			Total			Albumin			Globulin											
				In- crease	In- crease		In- crease	In- crease		In- crease	In- crease		In- crease	In- crease		In- crease		In- crease	In- crease	
			grams	grams	per cent	grams	grams	per cent	grams	grams	per cent	cm. H <sub>2</sub> O	cm. H <sub>2</sub> O	per cent	cc. H <sub>2</sub> O	cc. H <sub>2</sub> O	per cent		per cent	cm. H <sub>2</sub> O
W. H.	With- out vaso- dilatation	R	7.18			5.04			2.14			37.4			2990		45.8			13.1
		S	9.07	1.89	26.3	6.42	1.38	27.4	2.65	0.51	23.8	50.7	13.3	35.6	3080	90	53.0	7.2	15.7	122.2
	With vaso- dilatation	R	6.94			4.87			2.07			37.9			3000		44.0			12.7
		S	8.91	1.97	28.4	6.50	1.63	33.5	2.41	0.34	16.4	55.5	17.6	46.4	3140	140	52.0	8.0	18.2	124.7
J. P.	With- out vaso- dilatation	R	6.48			4.50			1.98			32.7			4300		40.6			10.4
		S	8.62	2.14	33.0	6.03	1.53	34.0	2.59	0.61	30.7	54.4	21.7	66.4	4435	135	51.7	11.1	32.1	122.7
	With vaso- dilatation	R	6.89			4.61			2.28			34.6			4295		39.9			10.0
		S	8.77	1.88	27.3	5.95	1.34	29.1	2.82	0.54	23.7	52.9	18.3	52.9	4425	130	47.8	7.9	19.8	124.2

The inconsistent results in the two subjects may have been due, in part, to variations in the length of the standing period, the vasomotor effects of venipuncture, sweating and the influence of syncope. Unfortunately the rigors of the experiment prevented the performance of a larger series, which might have avoided some of these difficulties.

### Summary

Heating of the forearms and hands was accompanied by a rise in the surface temperature of the feet (toe) with the subject erect, overcoming the drop in surface temperature which occurs in the quiet standing posture.

Vasodilatation resulted either in a greater or a lesser concentration of the blood in the feet and legs in the erect posture than occurs without vasodilatation. In either case the total volume of fluid filtered into the tissues of the leg appeared to be greater with vasodilatation.

### DISCUSSION

The results of these four studies indicate the essential difference in the mechanisms which tend

to limit the amount of fluid which accumulates in the dependent tissues during quiet standing and active exercise in the erect posture, respectively. In addition they emphasize the importance of the circulatory changes which accompany the assumption of the erect position. As was pointed out in the introduction, one of the primary factors concerned in limiting the loss of fluid from the blood in the standing-still position is an increase in the concentration of the blood in the dependent parts. This is aided by a decrease in the velocity and volume flow of blood in the feet and legs, shown by the studies of surface temperature and circulation time. The reduced velocity permits a greater loss of fluid per unit of blood with a greater local concentration and a higher colloid osmotic pressure, while the decreased flow limits the total amount of fluid filtered. On the contrary, in the moving leg the decrease in velocity does not occur, and the circulation time may be even shorter than in the reclining position. Some inability to maintain these changes in the circulation in the quiet standing position is indicated by the frequent occurrence of a secondary rise in the surface temperature as the subject continued to

stand, as well as by the fact that two subjects showed a much greater slowing of the circulation in the quiet leg during the first part of the standing period than later.

Bock, Dill and Edwards (7) have suggested that an increased circulation time in the standing-still position indicates, but does not prove, the existence of a smaller blood flow, since a great increase in the vascular bed might compensate for the slower circulation. Such a possibility is suggested by the great increase in size and number of open capillary loops in the erect posture. However, the great drop in surface temperature of the feet and legs in the standing-still position suggests that in spite of an increase in the vascular bed there is an actual decrease in blood flow as well as in the velocity of the circulation. The changes in the circulation represented by the drop in surface temperature and increased circulation time appear to be brought about, in a large measure at least, by a vasoconstriction, since vasodilatation prevented the drop in surface temperature which usually occurs on standing and presumably lessened the slowing of the circulation which occurs under the same conditions. This vasoconstriction does not appear to include the capillaries, although the latter seem still capable of constriction. Although the effect of vasodilatation on the concentration of the blood in the legs was variable, these results are believed to be consistent with the effect which vasodilatation might have under conditions of normal activity. Vasodilatation usually causes a more rapid blood flow. This should result in a smaller loss of fluid per unit of blood and hence less concentration of blood locally. At the same time, however, it causes a rise in capillary pressure, which tends to cause a greater filtration of fluid and hence a greater concentration of the blood. It appears, therefore, that in any given case the net result would depend on the relative importance of these two effects and might vary depending, among other things, on the degree and duration of vasodilatation. It seems possible that with maximum dilatation so much fluid might be filtered that blood flow would be significantly impeded by the increased viscosity, a state approaching true stasis (Landis). In this case the local concentration of blood would be greater than without vasodilatation. In other in-

stances, the influence of increased flow might predominate and cause less concentration of blood locally than occurs without vasodilatation.

As far as the total volume of fluid filtered into the tissues is concerned, it should be greater with vasodilatation (absence of vasoconstriction). Even though the more rapid blood flow in the latter case resulted in a smaller loss of fluid per unit of blood, the greater total flow should cause a larger total filtrate than occurs with vasoconstriction. Should the increased capillary pressure, occurring in the absence of vasoconstriction, cause a greater loss of fluid per unit of blood, the total filtrate would again be greater. In either case the conditions are similar to those existing during exercise in the erect posture. As we shall see, however, during exercise lymph drainage is able to remove the excessive filtrate, so that the volume of the leg increases but little or may even decrease. When vasodilatation occurs during quiet standing, lymph drainage is probably less able to remove the excess fluid, and the local accumulation would be greater than in the presence of the usual vasoconstriction.

In contrast to the standing-still position there is less concentration of the blood in the muscularly active leg in the erect posture. This suggests that less fluid is filtered from the blood in the erect posture when the legs are moving. Furthermore, the active leg showed a smaller increase in volume, which again suggests the filtration of a smaller amount of fluid. However, neither the lesser increase in the concentration of the blood nor the smaller increase in leg volume, in themselves, prove that a smaller amount of fluid was filtered from the blood into the tissues of the active leg. Moreover, all the evidence from other sources is to the contrary. Muscular activity is accompanied by a hyperemia, an increased intracapillary pressure and the production of osmotically active metabolic products which greatly increase filtration (10). According to Landis (11) fluid leaves the blood several times more rapidly during muscular activity than it does at rest under a venous pressure of 80 cm. of water. Under the conditions of our experiments, muscular activity and a high environmental temperature must have resulted in a greatly increased passage of fluid from the blood to the tissues. Although the

smaller increase in concentration of the blood in the "moving" leg indicates a smaller loss of fluid per unit of blood, a greater total blood flow would cause a greater total volume of filtrate. Furthermore, the smaller increase in volume of the "moving" leg, by limiting the rise in tissue pressure, would of itself permit a greater volume of fluid to be filtered. It would seem, therefore, that the smaller accumulation of fluid in the moving leg must be the result of a more rapid removal of the filtrate. This rapid removal is most probably accomplished by lymph drainage. It is known that muscular activity and an increased venous pressure cause an increase in the flow of lymph (12). At the same time they hinder resorption. An increased lymphatic drainage during muscular activity has been clearly shown in normal and edematous animals (dogs) by Weech, Goettsch and Reeves (13). In their experiments the onset of activity (walking) was accompanied by a greatly increased flow of lymph, which drained the leg of accumulated interstitial fluid and, in edematous dogs, gradually reduced or abolished the edema. Thus, with muscular activity, the excessive accumulation of fluid in the dependent parts during the erect posture is prevented, not so much by limitation of filtration as by the more rapid removal of an even greater volume of fluid than is filtered on quiet standing.<sup>5</sup> Since the volume of fluid removed by lymphatic drainage is presumably returned to the circulation rapidly, the reduction in total blood volume at any one time is probably much less than when standing still.

#### SUMMARY AND CONCLUSIONS

In summary, the exchange of fluid between the blood and tissues, primarily controlled by capillary and colloid osmotic pressure, is greatly influenced by a number of secondary factors, particu-

larly by posture. The great tendency to edema in the quiet erect posture is opposed by a rising colloid osmotic pressure and by an increasing tissue pressure in the feet and legs. These forces are aided by a decrease in the volume and velocity of the circulation in the legs. With muscular activity an even greater volume of filtrate than occurs on quiet standing is prevented from accumulating by a more active lymphatic drainage. Variations in these secondary factors will influence the exchange of fluid between the blood and the tissues and, in the presence of even slight changes in the serum proteins and capillary pressure, may determine the appearance or non-appearance of edema. Due consideration must be given to the influence of these secondary factors in interpreting the effect of abnormal variations in the fundamental forces concerned, especially under conditions of changing activity such as exist in ambulatory patients. For these reasons it is inadvisable to define too strictly the critical level of the serum proteins or venous pressure in relation to the pathogenesis of certain forms of edema.

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<sup>5</sup> Although the vasoconstriction and circulatory changes which we have discussed are most evident in the standing-still position and contribute greatly to the special mechanism by which the loss of fluid into the dependent tissues is limited in that position, they are undoubtedly effective during active movement as well and, under pathologic conditions especially, may modify the mechanism which controls the accumulation of fluid in the legs during exercise.



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# STUDIES ON THE IMMUNE RESPONSE OF THE RHEUMATIC SUBJECT AND ITS RELATIONSHIP TO ACTIVITY OF THE RHEUMATIC PROCESS. IV. CHARACTERISTICS OF STRAINS OF HEMOLYTIC STREPTOCOCCUS, EFFECTIVE AND NON-EFFECTIVE IN INITIATING RHEUMATIC ACTIVITY<sup>1</sup>

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The authors have reported (1) certain biological characteristics of strains of hemolytic streptococcus associated with pharyngeal infection, which appeared to reactivate the rheumatic process in individuals previously stigmatized with the disease. These organisms were in most instances *Streptococcus pyogenes*, according to Holman's classification; they produced skin toxin neutralizable by antiscarlatinal serum; they fell into six serological groups, and were indistinguishable from strains obtained from upper respiratory tract infection of non-rheumatic subjects. These studies also indicated that in many instances, perhaps 20 to 40 per cent, hemolytic streptococcus infection in individuals recognized to be rheumatic subjects is not followed by any clinical evidence of rheumatic activity. The failure of these patients to develop recrudescences suggested at least two possibilities: one, ineffectiveness of the strain; two, a refractory state of the host. The purpose of the present study was to determine, so far as possible with methods available, whether there are any demonstrable differences between those pathogenic agents which are effective and those which are non-effective in reactivating the rheumatic process.

Forty strains collected from 38 individuals comprise the material for the present study. These organisms were selected from individuals who had had at least one attack of rheumatic fever, who had been under close observation for a period of two to six years, who did not appear to be carriers of hemolytic streptococcus, who had been free of all clinical evidence of rheumatic activity for at least six months, and who con-

tracted, while being studied, a frank pharyngitis associated with the appearance of hemolytic streptococcus predominating in the throat flora. All of these infections occurred in the winter or spring months,<sup>2</sup> and the possibility of a rheumatic recrudescence was expected in each instance. Half of the individuals developed severe attacks of rheumatic fever. The organisms isolated from these patients at the time of pharyngitis are considered effective and are designated "E." In the other half, the rheumatic process appeared to remain quiescent, insofar as it was possible to determine with the methods now available.<sup>3</sup> The organisms isolated from the latter patients at the time of pharyngitis<sup>4</sup> were considered ineffective in initiating rheumatic activity and are designated "NE."

One of the first possible reasons to consider for the ineffectiveness of certain strains was that they might be of animal origin. Animal strains, as Lancefield (2) has demonstrated, show good

<sup>2</sup> The authors have observed that in New York City the hemolytic streptococcus throat infections occurring in the spring months have been more effective in initiating rheumatic activity than those in other seasons. A study of the toxin production of thirty strains of hemolytic streptococcus showed that of seven strains associated with fall infections, only one was a strong toxin producer whereas in the spring infections, about half of the organisms were strong toxin producers. This observation suggests the possibility that in New York City there may be a seasonal variation in the capacity of hemolytic streptococcus to produce soluble toxin as well as a seasonal variation in the incidence of these infections.

<sup>3</sup> These subjects were kept under close clinical and laboratory observation on the wards and in the out-patient department of the Presbyterian Hospital.

<sup>4</sup> These attacks of pharyngitis were well defined and in general more severe than those associated with the "E" infections.

<sup>1</sup> The work reported in this communication was carried out under The W. K. Kellogg Foundation.

agreement between their biochemical and cultural characteristics, and their serological classification. Therefore, a study of these biochemical characteristics was undertaken, to determine whether these strains were of animal or human type.

### TECHNIQUE

All cultures were seeded with 0.1 cc. of a 16 hour growth of the strain in phosphate neopeptone broth. Controls used throughout this work consisted of one strain known to give a positive, one strain known to give a negative reaction and also a tube of the medium inoculated with 0.1 cc. of sterile seed culture broth. The controls were incubated with the strains being tested. All of the organisms were beta hemolytic strains which caused marked hemolysis of rabbit red blood cells when grown on plates of blood agar or in tubes of blood broth.

The colony type was determined by the appearance on chocolate blood agar plates, following Todd's (3) technique. The plates were examined with a hand lens after

24 hours' growth, and the types were recorded as matt or glossy.

The method of Avery and Cullen (4) was used for determination of pH. Readings were made on the fourth day of cultures grown in 1 per cent dextrose broth. Brom cresol purple, methyl red and phenol red were used as indicators.

Fermentation reactions on trehalose and sorbitol were observed at the end of one week's incubation, according to the method of Edwards (5). All tubes were cultured to be sure of the presence of living organisms.

Reduction of methylene blue was tested with the method used by R. C. Avery (6). Readings were made daily for one week, and the final reading recorded.

Hydrolysis of sodium hippurate was tested as advocated by Ayers and Rupp (7). The reaction was considered negative when the hippurate and protein precipitates found at first were redissolved in ferric chloride leaving a clear solution.

From Table I it is seen that the pH of all strains falls between 5.1 and 5.7; that all ferment

TABLE I

*Biological and cultural characteristics of strains of hemolytic streptococcus isolated from the throats of rheumatic subjects during acute pharyngitis—E followed by acute rheumatism; NE followed by good health*

Strain	Colony type	pH in dextrose broth	Fermentation of		Methylene blue tolerance	Hydrolysis of sodium hippurate	Fermentation of sugars			Sugar fermentation type	Enzyme production				Streptolysin production*	Erythro-genic toxin production
			Sorbitol	Trehalose			Lactose	Mannite	Saline		Liquefaction of gelatin	Digestion of casein	Histase activity	Fibrinolytic activity		
E 18	Matt	5.3	—	+	—	—	+	—	+	Pyogenes	—	—	Weak	+	.000120	Strong
E 35	Matt	5.4	—	+	—	—	+	—	+	Pyogenes	—	—	Weak	++++	.000008	Strong
E 36	Matt	5.3	—	+	—	—	+	+	+	Infrequens	—	+	Weak	++	.000008	Strong
E 38	Matt	5.3	—	+	—	—	+	—	+	Pyogenes	—	+	Weak	++++	.000024	Strong
E 45	Matt	5.1	—	+	—	—	+	—	+	Equi	—	—	Negative	++	.000003	Strong
E 46	Matt	5.3	—	+	—	—	—	+	+	Hemolytic II	—	—	Moderate	++++	.000003	Negative
E 47	Matt	5.3	—	+	—	—	+	—	+	Equi	—	+	Weak	++++	.000008	Strong
E 50	Matt	5.7	—	+	—	—	+	—	+	Pyogenes	—	+	Weak	++	.000030	Strong
E 51	Matt	5.6	—	+	—	—	+	—	+	Pyogenes	—	+	Moderate	+	.000020	Weak
E 57	Matt	5.3	—	+	±	—	+	—	+	Pyogenes	—	+	Strong	+	.000003	Strong
E 65	Matt	5.1	—	+	—	—	+	—	+	Pyogenes	—	+	Weak	+++	.000003	Strong
E 66	Matt	5.3	—	+	—	—	+	—	+	Pyogenes	—	+	Strong	+++	.000003	Weak
E 67	Matt	5.3	—	+	—	—	+	—	+	Pyogenes	—	+	Weak	+++	.000020	Strong
E 69	Matt	5.3	—	+	—	—	+	—	+	Equi	—	—	Weak	++	.000020	Strong
E 80	Matt	5.5	—	+	—	—	+	—	+	Pyogenes	—	—	Negative	+++	.000004	Strong
E 83	Matt	5.1	—	+	—	—	+	—	+	Pyogenes	—	—	Weak	++++	.000003	Strong
E 90	Matt	5.3	—	+	—	—	+	—	+	Pyogenes	—	+	Strong	++++	.000003	Strong
E 91	Matt	5.3	—	+	—	—	+	—	+	Pyogenes	—	+	Weak	+	.000020	Strong
E125	Matt	5.2	—	+	—	—	+	+	+	Infrequens	—	±	Negative	+	.000004	Strong
EpEH	Matt	5.4	—	+	—	—	+	—	+	Pyogenes	—	—	Moderate	++++	.000003	Strong
NE 1	Glossy	5.3	—	+	—	—	—	—	—	Subacidus	—	—	Weak	++++	..	Weak
NE 2	Matt	5.7	—	+	—	—	+	—	—	Anginosus	—	—	Weak	++++	..	Negative
NE 4	Glossy	5.8	—	+	—	—	+	—	—	Anginosus	—	—	Weak	+	.000001	Negative
NE 5	Matt	5.3	—	+	—	—	+	—	+	Pyogenes	—	+	Weak	++++	..	Negative
NE 6	Matt	5.2	—	+	—	—	+	—	+	Pyogenes	—	—	Weak	++	..	Strong
NE 7	Matt	5.3	—	+	—	—	+	—	+	Pyogenes	—	+	Weak	++++	.000001	Negative
NE 8	Glossy	5.1	—	+	+	—	+	—	+	Equi	—	—	Weak	++++	..	Negative
NE 9	Glossy	5.3	—	+	—	—	+	—	+	Anginosus	—	±	Weak	++	.000004	Negative
NE 10	Matt	5.2	—	+	—	—	+	—	+	Pyogenes	—	+	Weak	++++	.000030	Negative
NE 11	Matt	5.1	—	+	—	—	+	—	+	Equi	—	—	Weak	++	.000024	Negative
NE 12	Matt	5.6	—	+	—	—	+	—	+	Pyogenes	—	+	Moderate	+	..	Negative
NE 13	Matt	5.3	—	+	—	—	+	—	+	Pyogenes	—	+	Weak	++++	..	Weak
NE 14	Matt	5.3	—	+	—	—	+	—	+	Pyogenes	—	+	Weak	++++	..	Negative
NE 15	Matt	5.1	—	+	—	—	+	+	+	Infrequens	—	—	Weak	+	.000004	Strong
NE 16	Matt	5.1	—	+	—	—	+	—	+	Pyogenes	—	+	Weak	++++	..	Weak
NE 17	Matt	5.2	—	+	—	—	+	—	+	Equi	—	±	Weak	—	..	Strong
NE 18	Matt	5.1	—	+	—	—	+	—	+	Pyogenes	—	—	Negative	++++	..	Strong
NE 19	Matt	5.1	—	+	—	—	+	—	+	Equi	—	+	Negative	++	.000004	Negative
NE 20	Matt	5.2	—	+	—	—	+	—	+	Pyogenes	—	+	Weak	++++	..	Negative
NE 21	Matt	5.2	—	+	—	—	+	—	+	Pyogenes	—	+	Weak	++	..	Negative

\* Volume of Todd's globulin required to neutralize 1 M.H.D. of streptolysin.

\*\* Streptolysin titer too weak to be determined.

trhalose; that none ferment sorbitol; that only one, or possibly two, reduce methylene blue; that none hydrolyze sodium hippurate. These findings indicate (see Lancefield (2)) that all the strains in both the E group and the NE group are of the human type.

Previous studies (1) have shown that 20 out of 27 strains of organisms associated with acute rheumatism were of the *Streptococcus pyogenes* group (S); that is, they fermented lactose and salicin but not mannite. In order to determine whether these characters were of any significance in determining the effectiveness of a strain in reactivating the rheumatic process, these same fermentation reactions were tested with E and NE strains and also with 33 strains obtained during the same period of time from non-rheumatic subjects with acute pharyngitis. The results of the test on rheumatic subjects, detailed in Table I, are summarized, along with the data on non-rheumatic controls, in Table II. There was no

TABLE II

*Types of hemolytic streptococcus obtained from cases of pharyngitis in rheumatic and non-rheumatic subjects*

Source of organism	Pyogenes	Equi	Hemolytic III	Inferquens	Anginosus	Hemolytic II	Hemolytic I	Sub-acides
Rheumatic subjects:								
E strains..	14	3		2		1		
NE strains..	11	4		1	3			1
Non-rheumatic subjects..	20	6	2	2	1	1	1	

significant difference in any of the three groups of organisms. During this period of study *Streptococcus pyogenes* has been the prevalent variety associated with throat infections in New York City.

Another series of tests was undertaken to determine whether there are differences in the capacity of the two groups (E and NE) to digest proteins. The liquefaction of gelatine, hydrolysis of casein, tissue digestion by histase, and plasma clot dissolution by fibrinolysin (9, 10) were investigated.

*Technique—gelatin digestion.* The organisms were grown in 10 per cent gelatin for seven days at room temperature and liquefaction was sought but in no instance observed.

*Casein digestion.* Plates were made with 1 cc. sterile milk being added to 12 cc. agar pH 7.4. Three heavy inoculations from 16 hour old colonies were streaked across the surface of a plate which was then incubated for 72 hours at 37° C. The plates were flooded with 12 per cent acetic acid and digestion indicated by the appearance of a permanent clear zone around the colonies. One plate was used for each strain tested.

*Tissue-digesting enzyme.* Histase detection was made according to Holman's (11) modification of Robertson's cooked-meat medium. Digestion was determined by observing the subsidence of meat in the tubes. Readings were made according to Frobisher (12) after 5 days in the incubator at 37° C. The amount of digestion was measured in millimeters.

*Fibrinolysin.* The method of Tillett and Garner (9) was followed. The material used consisted of 0.2 cc. of fresh plasma from one individual (previously determined to be readily dissolved by fibrinolysin), 0.8 cc. saline, 0.5 cc. filtrate of organism tested, 0.25 cc. of a 0.25 per cent solution of CaCl<sub>2</sub> in 0.85 per cent saline. The tubes were incubated in a water bath at 37° C. Complete dissolution in less than 30 minutes is recorded as ++++ fibrinolytic activity; 30 to 60 minutes, +++; one to four hours, ++; 4 to 24 hours, +; incomplete dissolution in 24 hours, —.

The results are to be found in Table I. No significant differences in enzyme production were found between the two groups. Both failed to liquefy gelatin; a few of each group digested casein; histase production seemed perhaps more marked among a few E strains; and many strains of each group were strong in fibrinolytic activity.<sup>5</sup>

*Streptolysin production.* The forty organisms under investigation were tested in this laboratory for streptolysin production. In addition, nine organisms isolated from the throats of rheumatic subjects in New York were tested in England by Dr. Todd. His findings are presented in Table III.

*Technique for determining streptolysin productions.* The material and technique was identical with that described for making and titrating streptolysin in the first paper of this series (27). The

<sup>5</sup> Sera from these patients were tested for antifibrinolysin according to the method of Tillett and Garner (9). Four groups were studied: rheumatic subjects infected with E strains; rheumatic subjects infected with NE strains; patients with acute rheumatism, the infectious agent being undetermined; non-rheumatic subjects convalescing from proven hemolytic streptococcus infections. No significant differences were found. The results were in accord with the recent observations of Hadfield et al. (28) and Myers et al. (29).

TABLE III

Hemolytic titers and volumes of antistreptolysin required to neutralize 1 M.H.D. as determined by Doctor Todd

Strains from hemolytic streptococcus pharyngitis	Character of rheumatic attack	pH of filtrate	Hemolytic titer		Volume of antistreptolysin required to neutralize 1 M.H.D. of hemolysin
			Complete hemolysis	Partial hemolysis	
E 65.....	Severe	6.6	cc. 5.0	cc. 0.50	0.00003
E 67.....	Severe	6.7	1.0	0.05	0.00003
E 69.....	Severe	7.1	1.0	0.05	0.00004
E 64.....	Mild	6.8	2.0	0.05	0.00004
E 80.....	Mild	7.0	1.0	0.05	0.00005
E 84.....	Mild	7.0	0.5	0.02	0.00005
NE 25.....	None	5.6	None	None	
NE 26.....	None	7.0	None	None	
NE 27.....	None	7.9	None	None	

TABLE IV

Sample arrangement of tubes†

Strain	Lysins, cc.	Hemolytic power*							
		2	1	.75	.5	.4	.3	.2	.1
E 50.....		C	C	PC	PC	PC	PC	PC	VM
NE 9.....	VM	M	M	S	S	VS	VS	—	—
NE 7.....	—	—	—	—	—	—	—	—	—
Contents of tubes	Lysins 2 cc., 1 cc., etc. Cells 0.5 cc.								
		Combining power							
E 50.....		C	VM	—	—	—	—	—	—
NE 9.....	—	—	—	—	—	—	—	—	—
NE 7.....	—	—	—	—	—	—	—	—	—
Contents of tubes	Globulin 1/20,000 dose = 1 cc. in each tube Lysin 2 cc., 1 cc., etc. Cells 0.5 cc.								
Strain	Globulin, cc.	Antistreptolysin required							
		1	.9	.8	.7	.6	.5	.4	.3
E 50.....		—	—	—	—	VS	S	M	VM
NE 9.....	—	—	—	—	—	—	—	—	—
NE 7.....	—	—	—	—	—	—	—	—	—
Contents of tubes	Globulin 1 in 10,000 = 1 cc., 0.9 cc., etc. Lysin undiluted 0.5 cc. in each tube Cells 0.5 cc.								

\* C = complete; VM = very marked; M = marked; S = slight; VS = very slight; — = none.  
† All tubes containing less than 2 cc. are brought up to 2 cc. volume with .85 per cent saline.

hemolytic power, the combining power and the antistreptolysin necessary for neutralization were determined. The tubes were set up as in the sample tabulations in Table IV.

The results of the tests on the forty strains are to be found in Table I. It is seen that streptolysin production in glucose bicarbonate broth occurred both with E and NE organisms. However, this activity was more marked in the group of E strains. All but two of these organisms produced strong lysins. This was in accord with the findings of Todd (13). Only seven of the NE strains produced strong lysins; in the remaining thirteen the streptolysins were too weak for serological examination.

The capacity of these organisms to produce streptolysin in man was tested by determining the antistreptolysin titers in patients infected with these E and NE strains. In 25 members of this group it was possible to obtain blood serum just before or at the onset of pharyngitis and to make serial examinations during the two months after infection. The maximum antistreptolysin titers reached by the individuals infected with E and NE strains are presented in Table V, along with the organisms' streptolysin titers in glucose bicarbonate broth.

The findings in these 25 patients, divided into two classes, may be seen in Table V. All of the "E" infections were followed by striking increases in the patients' antistreptolysin titers. None of the "NE" infections were followed by more than a slight rise in titer level. The difference between the mean  $\Delta \log u$  of the E group and the mean  $\Delta \log u$  of the NE group is 0.74 minus 0.06 = 0.68, with a probable error of 0.074. The difference between the two means is therefore 9.2 times its probable error  $\left(\frac{0.68}{0.074} = 9.2\right)$ .

This shows that the increase of titer in the E group is significantly greater than that in the NE group. The marked change in antistreptolysin level of the first class of patients may be attributed to the strong streptolysin production of the infecting organisms. The slight change in antistreptolysin level of the second class may be attributed to the weak streptolysin production of most of the infecting strains. However, in four cases the streptolysin production (in vitro) of the

TABLE V

*Change in antistreptolysin titer following pharyngitis with E and NE strains of hemolytic streptococcus*

Patient	Organism	Streptolysin titer of culture filtrate (amount of Todd's antistreptolysin required to neutralize 1 M.H.D. of streptolysin)	Antistreptolysin titer of patient		Logarithm of titer = log $\mu$		
			At time of infection	Following infection	At time of infection	Following infection	$\Delta$ log $\mu$
		<i>cc.</i>	<i>units</i>	<i>units</i>			
O'Hare.....	E 35	.000008	50	333	1.70	2.52	.82
Hanke.....	E 38	.000024	50	200	1.70	2.30	.60
Rothenberg.....	E 45	.000008	333	500	2.52	2.70	.22
Servetman.....	E 47	.000008	33	250	1.52	2.40	.88
Hallahan.....	Ep E11	.000008	200	1250	2.30	3.10	.80
Terentino.....	E 50	.000030	166	500	2.22	2.70	.48
Cerney.....	E 51	.000020	33	1250	1.52	3.10	1.58
Aita.....	E 65	.000008	14	125	1.16	2.10	.94
Riccardi.....	E 67	.000020	83	250	1.92	2.40	.48
Gilligan.....	E 69	.000020	33	333	1.52	2.52	1.00
Clowry.....	E 80	.000004	125	500	2.10	2.70	.60
Weber.....	E 90	.000008	166	250	2.22	2.40	.22
Maurer.....	E 125	.000004	250	2500	2.40	3.40	1.00
			100 (median)	333 (median)	1.91 (mean)	2.64 (mean)	+.74 (mean)
Solomon.....	NE 4	.000004	33	33	1.52	1.52	.00
Rodriguez.....	NE 5	*	50	62	1.70	1.80	.10
Delaney.....	NE 6	*	62	83	1.80	1.92	.12
Bourie.....	NE 10	.000050	143	143	2.16	2.16	.00
Jackson.....	NE 13	*	71	143	1.85	2.16	.31
Pisillo.....	NE 14	*	125	143	2.10	2.16	.06
Fromer.....	NE 15	.000004	250	333	2.40	2.52	.12
Miller.....	NE 17	*	100	111	2.00	2.05	.05
McFarland.....	NE 18	*	143	143	2.16	2.16	.00
Tollerton.....	NE 19	.000004	333	500	2.52	2.70	.18
Skea.....	NE 20	*	250	125	2.40	2.10	-.30
Zelesney.....	NE 21	*	125	166	2.10	2.22	.12
			125 (median)	143 (median)	2.06 (mean)	2.12 (mean)	+.06 (mean)

\* Streptolysin content too low to be titrated.

infecting strains was high. In these cases the absence of a significant rise in antistreptolysin titer was presumably not due to lack of streptolysin. Whether it may be attributed to a weak response of the host is to be considered in detail in the following papers of this series.

*Production of skin toxin.* Previous studies (1) have indicated that the organisms associated with acute rheumatism are erythrogenic; that is, they produce capillary toxin which causes an erythematous skin reaction when injected intracutaneously into Dick positive individuals or into full-grown Silver Fox rabbits.

The toxin production of the forty organisms was studied by testing the capacity of each filtrate to produce an erythematous reaction. The filtrate was made from a forty-eight hour growth in Difco proteose broth. The skins of two Silver

Fox rabbits which had previously been found to give a 1 cm. reaction to 5 S.T.D. (skin test doses) of Dick toxin were used for each test. Dilutions of filtrate 1:50 and 1:100 were injected intracutaneously. Controls consisted of broth and heated filtrate. When the results were questionable, the filtrates were tested on other animals. The reactions are expressed as follows: no reaction, negative; macular lesion over 1 cm. in diameter, weak; a papular lesion with erythema more than 1.5 cm. in diameter, strong. All readings were made at twenty-four hours. The findings are presented in Table I.

It is seen in Table I that sixteen out of twenty E strains were strong toxin producers, and in only one instance was this function found lacking. In contrast, of twenty NE strains only four were strong toxin producers, and in thirteen no

soluble toxin could be detected. The correlation between intensity of the rheumatic attack and strength of toxin production previously reported (1) seemed to be present in this study.

#### DISCUSSION

The relationship between scarlatina and rheumatic fever is an interesting one. Epidemiological observations indicate a similar distribution. In the North Temperate Zone where scarlet fever is prevalent, acute rheumatism is common and severe. In contrast, in tropical environments where scarlet fever is absent (14), acute rheumatism appears to be rare and mild. That streptococcus infection of the tonsils occurs in the tropical environment of Puerto Rico has been shown by Pomales (15). A limited study of Pomales strains has been made by the authors. This small group of organisms did not produce skin toxin, were not pathogenic for young rats and were antigenically different from scarlatinal strains and from organisms effective in initiating rheumatic recrudescences. In an extensive study of hemolytic streptococcus in British Guiana, Grace and Grace (16) have demonstrated that strains of hemolytic streptococcus isolated from abscesses and lymphangitis, either did not produce skin toxin or produced a toxin not neutralizable by scarlatinal antitoxin. These organisms were serologically unrelated to strains obtained from patients with scarlet fever in England and in New York. According to Grace (17) "they differ markedly from the common beta hemolytic streptococcus of temperate climates in morphology, serological reactions and in virulence for man and mice." It is conceivable that the rarity of acute rheumatism in the Tropics and the parallel absence of scarlatinal infection may be due to inhibited activity of *Streptococcus hemolyticus* as a respiratory pathogen.

Clinicians have long recognized that scarlet fever in children is sometimes followed after a period of weeks by an initial attack of acute rheumatism. Paul et al. (18), studying post-scarlatinal rheumatism in New Haven, found "that many examples of post-scarlatinal rheumatism or carditis either represent the lighting up of a previously unsuspected latent or sub-clinical form of rheumatic fever, or represent the manner

in which an individual possessed of a 'rheumatic diathesis' may react to scarlet fever." The present authors have observed both initial rheumatic attacks following scarlatina and the development of recrudescences in rheumatic subjects who probably had scarlet fever without a rash (19). These rheumatic patients were infected with organisms that caused scarlet fever in their siblings.<sup>6</sup>

As far as is known, the rheumatic patient only rarely develops the clinical picture of scarlet fever. Hector (20), studying scarlet fever in England, was able to elicit evidence of previous rheumatism in only a few of a large group of scarlatinal patients. A study of the discharge diagnoses of patients at Willard Parker Hospital, New York City, showed that the incidence of mitral stenosis in individuals admitted with scarlet fever was extremely low. Furthermore, although throat infections occur frequently during the winter and spring months, only three times has scarlet fever been seen by the authors<sup>7</sup> in a group of more than 500 rheumatic children during a period of six years.

The immunological evidence offers a possible explanation for the rarity of scarlet fever in rheumatic subjects. According to the collected statistics of the Pickett-Thomson Research Laboratory (21), approximately 50 per cent of urban school children under fifteen years of age are Dick positive. On the other hand, Swift et al. (22), while studying the reaction to the Dick test in children with rheumatic fever, found that among 112 patients with either active or latent rheumatic fever, only fifteen showed positive reactions.

<sup>6</sup> An illustrative example follows: Katherine H. and William H., sister and brother, had been under observation in the Vanderbilt Clinic for over two years when both contracted a throat infection on the same day. Katherine, who was known to be a rheumatic subject with mitral stenosis, recovered from pharyngitis and remained symptom-free for ten days. William, who in the past had had neither symptoms nor signs of rheumatism, developed the typical clinical picture of scarlet fever the day after infection of the throat. He was placed in isolation at Willard Parker Hospital. Two weeks later both children developed epistaxes, fever and joint pains. Katherine (History No. 417904) was admitted to Babies Hospital with a rheumatic recrudescence and William (History No. 294788) was admitted to Babies Hospital with his initial rheumatic attack, accompanied by severe carditis.

<sup>7</sup> Two cases were followed by pancarditis, the third by mild carditis.

Kaiser (23), Collis (24) and other workers have likewise observed a low incidence of Dick positive reactions in children with rheumatism. Among the authors' group of rheumatic patients under the age of fifteen, less than 10 per cent were Dick positive, although less than 5 per cent of the group gave a history of having had scarlet fever. The high incidence of Dick negative reactions among rheumatic children who have not had scarlet fever points towards experience with erythrogenic organisms, presumably in the course of frequent throat infections with E strains. This would account for the rarity of the clinical picture of scarlet fever among children with rheumatic heart disease. The small percentage, less than ten, of Dick positive rheumatic patients corresponds to the 10 per cent of non-rheumatic patients who fail to become Dick negative after scarlet fever.

Finally, there appears to be a close similarity between the type of hemolytic streptococcus associated with scarlatina and rheumatic fever. The organisms recovered from the throats during scarlet fever and the throat strains effective in initiating recrudescences in the rheumatic subject are serologically indistinguishable (1, 25). They are both characterized by their capacity to elaborate soluble toxin (1) and streptolysin (13). They both stimulate antibody production in high titer as judged by the antistreptolysin levels developed in individuals recovering from scarlet fever or individuals with acute rheumatism (26). The patient with rheumatic disease is usually Dick negative and fails to develop the rash of scarlet fever when infected with a scarlatinal strain. This was striking in The Pelham Home epidemic, described in preceding papers of this series (27). At that time a highly effective agent<sup>8</sup> (Strain EpEH, Table I) was found to produce strong scarlatinal toxin, to cause pharyngitis without a rash in rheumatic subjects, and to give rise to a severe outbreak of rheumatic recrudescences.

## SUMMARY

The biological characteristics of strains of hemolytic streptococcus associated with acute pharyngitis in rheumatic subjects have been studied.

Half of the strains selected initiated intense activity of the rheumatic process and the other half failed to initiate rheumatic activity in susceptible subjects.

The cultural characteristics of the organisms studied were essentially the same in both groups. All strains were of human type.

The effective organisms were characterized by the capacity to produce strong skin toxins and streptolysins and were indistinguishable from scarlatinal strains of *Streptococcus hemolyticus*. They gave rise to the development of high titers of antistreptolysin in the subjects infected.

Those strains which failed to produce skin toxin and streptolysin, and did not give rise to the development of high titers of antistreptolysin, were ineffective in activating the rheumatic process.

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<sup>8</sup> The strains from this epidemic fell into our group VI (1), Griffith Type 26 (25). It is possible that the typing of hemolytic streptococcus may help to determine which organisms are highly effective in initiating rheumatic activity.



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# STUDIES ON THE IMMUNE RESPONSE OF THE RHEUMATIC SUBJECT AND ITS RELATIONSHIP TO ACTIVITY OF THE RHEUMATIC PROCESS. V. ACTIVE AND PASSIVE IMMUNIZATION TO HEMOLYTIC STREPTOCOCCUS IN RELATION TO THE RHEUMATIC PROCESS<sup>1</sup>

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There seems to be a close relationship between infection with hemolytic streptococcus, producing skin toxin, and initiation of rheumatic activity in susceptible subjects. The failure of the rheumatic patient to develop activity of the disease process when infected with a non-erythrogenic strain of hemolytic streptococcus suggests the possibility that toxin may play an important rôle in the genesis of rheumatic lesions. To test the possibility that an increase in circulating antitoxin to streptococcus might protect the tissues of the rheumatic subject and modify the disease attack, the authors have made two studies, one on active immunization with scarlatinal (NY5) toxin, the other on passive immunization with NY5 antitoxin.

## I. ACTIVE IMMUNIZATION WITH SCARLATINAL TOXIN

### *Its relation to streptococcus infection and rheumatic fever*

Reports on the effectiveness of active immunization with streptococcus toxin vary widely. Most observers agree that the procedure prevents scarlet fever and many consider it effective in lowering the incidence of throat infections and their complications, such as rheumatic fever and nephritis. The literature has been summarized by the Pickett-Thomson Research Laboratory (1). The present study has been made to determine whether active immunization with streptococcus toxin may increase resistance to infection and whether it may modify the host's reactivity.

### *Character of the group under observation*

The individuals under observation consisted of two classes of student nurses entering training at the Presbyterian Hospital in 1932 and in 1933. Half of each class was immunized and the other half was observed as a control group. The subjects were in good health, had not experienced recent streptococcus infection, had neither history nor signs of rheumatic disease and had in most instances lived in the vicinity of New York.

### *Method of immunization*

Fifty-two members of one class and 61 members of the other were immunized with NY5 streptococcus toxin. The material was purified and concentrated by Dr. Michael Heidelberger. Injections were given twice a week, beginning with 500 S.T.D. (skin test doses) and reaching a maximum of 80,000 S.T.D., after a period of two or three weeks. Each individual received between 300,000 and 400,000 S.T.D.

### *Results*

The results of immunization in comparison with the control group may be considered from three standpoints: (a) alteration of skin reactivity; (b) influence on the incidence of respiratory infection; (c) effect on the development of rheumatic fever and nephritis.

(a) *Alteration of skin reactivity.* The change in skin reactivity after immunization with NY5 toxin is seen in Table I. Before immunization, the majority of individuals were Dick positive. With one exception, all were Dick negative following immunization and remained so over a period of one year. About 80 per cent became skin negative to 20 S.T.D. following immuniza-

<sup>1</sup> The work reported in this communication was carried out under The W. K. Kellogg Foundation.

TABLE I

*Skin reactions to streptococcus toxin before and after immunization*

	Group A—1932				Group B—1933			
	Skin reactive to			Number tested	Skin reactive to			Number tested
	1 S.T.D.	5 S.T.D.	20 S.T.D.		1 S.T.D.	5 S.T.D.	20 S.T.D.	
Before immunization....	29	44	50	52	30	37	44	61
One month after immunization....	2	3	19	52	0	0	8	61
Four months after immunization....	1	8	21	50				
One year after immunization....	1	8	21	50				

tion. Skin reactivity decreased markedly in all but three individuals.

(b) *Influence on the incidence of respiratory infection.* A clinical record was kept on each individual and every respiratory infection was reported to the physician in charge. Cultures of the throat were made on these occasions. During the period of observation the immunized group of approximately 100 individuals contracted 24 respiratory infections associated with hemolytic streptococcus.<sup>2</sup> Nearly all of these individuals had negative skin tests to 20 S.T.D. In the control group there were nineteen similar infections and one case of scarlet fever.

(c) *Influence on the development of sequelae.* Both the control and immunized groups included only selected individuals, as all known rheumatic subjects had been removed. Nevertheless, two individuals in the immunized group developed rheumatic carditis and one severe nephritis following cervical adenitis. Likewise in the control group there was one instance of acute rheumatism and one of acute nephritis among the nineteen throat infections.

In brief, the findings indicate that although skin reactivity to streptococcus toxin was diminished by

active immunization,<sup>3</sup> there was no evidence that it increased resistance to streptococcus infection or prevented the development of rheumatic disease.

## II. PASSIVE IMMUNIZATION WITH ANTISTREPTOCOCCUS SERUM

Two findings have been almost constantly associated with activation of the rheumatic process: first, that the effective (see Paper IV of this series (2)) organisms produce strong soluble toxins, and second, that the host develops an antibody response (antistreptolysin) at the time of onset of rheumatic activity. Whether toxin is itself important in initiating rheumatic disease or whether toxin production in vitro is merely an index of a highly active strain is unknown. It seemed possible that the introduction of streptococcus antitoxin during respiratory infection and during the symptom-free phase before the onset of an expected rheumatic attack, might throw light on the rôle played by toxin in the initiation of rheumatic activity.

### *Group of patients studied*

Ten patients were selected for this study, including seven males and three females. Their ages were between fourteen and thirty. All were highly susceptible rheumatic subjects with varying degrees of cardiac damage who had been under observation for a period of two to six years. Six of the group, while under the authors' care, had previously contracted hemolytic streptococcus infections which had been followed by severe attacks of rheumatic fever. None had experienced streptococcus disease, so far as could be determined, for at least one year. Two throat cultures had been taken on each individual every month. In each individual the rheumatic process appeared to be quiescent at the beginning of the study. All gave negative skin tests to horse serum and were in excellent condition at the onset of pharyngitis. Each individual reported the symptoms of pharyngeal infection at the onset of the respiratory infection. Throat cultures at that time showed hemolytic streptococcus in predominance. Each subject was admitted to the wards

<sup>2</sup> These organisms were all beta strains of hemolytic streptococcus. Some produced toxins which were neutralized by NY5 antitoxin.

<sup>3</sup> Skin reactivity to streptococcus nucleoprotein was not influenced.

of the Presbyterian Hospital with the characteristic clinical picture of acute pharyngitis.

### Procedure

The patients were placed in bed in the hospital and the local infections were treated in the customary manner, with the exception that no salicylates were given because of the possibility of masking rheumatic symptoms. Antistreptococcus serum was given to some of the patients during the period of pharyngitis; to others at the time the pharyngitis was subsiding and to the remainder during the quiescent interval, after infection but before rheumatic manifestations were due to appear. The material used was NY5 antiserum, lot number 223B, supplied especially for this purpose by the New York State Department of Health. This antiserum was chosen because of its broad valence and because it had previously been shown (2) to neutralize, in about 70 per cent of instances, the toxins formed by organisms effective in initiating rheumatic activity in New York City. The serum was fresh, contained 800 units of antitoxin per cc. and was kept at 4° C. during this study. At the beginning of immunization small quantities were diluted with saline and given intravenously. The doses were increased in the customary manner from .01 cc. to .1 cc. to 1 cc. to make sure that the patient could tolerate 10 cc. without symptoms. Only one patient experienced any distress, and in this individual (Patient G.S.) administration of serum was discontinued. The others received antitoxin each day for five days, the total dosage varying between 40,000 and 100,000 units. The usual precautions against anaphylaxis were observed with great care. Nevertheless, two individuals of the group, although skin tests were negative and although they had not received horse serum in the past, developed severe serum disease a few days after their last dose of antitoxin.

The organisms associated with the throat infections were studied shortly after isolation. Their capacity to produce soluble toxin was tested on at least two adult silver fox rabbits in the manner used throughout these studies (2). The filtrates which caused severe reactions in dilutions 1:200 or greater were considered strong toxin producers

(+++ or ++++); those which produced 1 to 2 cm. reaction in dilution 1:50, weak toxin producers (+); those which caused no reaction in dilution 1:10 were considered negative. Negative strains were checked by making a second and in some cases a third group of filtrates. Neutralization tests were carried out in the usual manner by incubation with an appropriate amount of NY5 antitoxin at 37° C.

Blood serum was obtained under sterile conditions from each individual before the administration of any antitoxin. Samples were similarly collected shortly after the last dose and subsequently at weekly intervals. All of the specimens were stored at 4° C. and the antistreptolysin titers were determined at the completion of the experiment.

Following the administration of serum each patient was kept in bed. Electrocardiographic tracings, white blood counts and blood sedimentation rate determinations were made twice a week. Those individuals who appeared to escape recrudescences after three weeks in bed were observed in the Out-patient Department where the laboratory studies were repeated. Tables II and III, illustrative of the records kept on each subject, and their histories, are presented in brief.

### CASE HISTORIES

*C. G., Number 238300.* The patient was a boy of fifteen who had been under observation for two years. He had experienced a typical attack of rheumatic fever at six years of age and developed mitral stenosis. In 1932 and 1933 he was in excellent health and symptom-free until the onset of pharyngitis on March 9th. He was admitted to the Presbyterian Hospital on the first day of his infection and given 90,000 units of antiserum. Clinical observations and laboratory findings are presented in Table II. This table is divided into three phases—acute pharyngitis, symptom-free interval, attack of acute rheumatism. The findings are similar to those in the other patients who developed recrudescences. During mild serum sickness the blood sedimentation rate fell to a strikingly low level.<sup>5</sup> This rose on the tenth day after pharyngitis, and marked leukocytosis appeared. The antistreptolysin titer rose rapidly, and on the fourteenth day he developed severe rheumatic carditis beginning with abrupt development of pyrexia. Rheumatic activity persisted for at least three months (see Table II). The polyarthritis was more intense than he had ever experienced.

<sup>5</sup> The blood sedimentation rate has been observed to be low during periods of serum sickness with edema.

<sup>4</sup> It also contained 4,000 units of antistreptolysin per cc.

TABLE II

*Data on Patient C. G., Number 238,300*

Disease stage	Date	Blood			Throat flora	Clinical observations	Remarks *
		W.B.C.	Sedimentation rate	Anti-streptolysin titer			
	1933		mm.	units per cc.			
Phase 1 Acute hemolytic streptococcus infection	March 9	15,000			Hemolytic streptococci predominant Hemolytic streptococci predominant	First day of acute pharyngitis. Temperature 101°	Ekg. T2 is diphasic
	March 10	15,400				Acute pharyngitis. Temperature 101°	NY5 serum number 223B 10,000 units
	March 11		16	33		Subsiding pharyngitis	NY5 serum number 223B 20,000 units
Phase 2 Symptom-free interval	March 12				Hemolytic streptococci disappearing	Good condition. Apparently quiescent	NY5 serum number 223B 20,000 units
	March 13		8				NY5 serum number 223B 20,000 units
	March 14						NY5 serum number 223B 20,000 units
	March 15						
	March 16		4	33		Serum sickness. Mild urticaria	Ekg. normal. Blood sedimentation rate perhaps depressed by serum sickness
	March 17						
	March 18					Nausea and vomiting. Sore throat and general glandular enlargement	
	March 20	16,900					
	March 21						
	March 22						
	March 23	18,550	34			Good health. Apparently quiescent	
	March 24	21,550					
	March 25	22,350		111			
Phase 3  Moderately severe rheumatic attack	March 27	19,200		200		Acute rheumatism. Temperature 103°. Carditis and polyarthritis	Ekg. T2 diphasic
	March 28	21,800	66				Aspirin started
	March 29					Continued activity of the rheumatic process	
	March 30	13,900	124				Ekg. normal
	March 31						Aspirin stopped
	April 4	9,420	115	333			Ekg. normal
	April 7						Aspirin started
	April 28	9,800	64			Polyarthritis	
	April 11	9,350	86			Persistent activity—symptoms suppressed by salicylates	Hemoglobin loss 30 per cent. R.B.C. loss 1,000,000. Ekg. normal. Discharged for convalescent care
	May 15			250			Sent to Reed Farm and had recrudescence one week later
Good health	November 9 1934			125		Marked improvement. Symptom free	
	January 13			83		Good health, symptom free	

\* Ekg. = electrocardiogram.

*M. A., Number 63035.* The patient was a girl of twenty who had been under observation for eight years. During this period she had three rheumatic attacks without polyarthritis. All of these followed throat infections with hemolytic streptococcus. She had developed advanced heart disease but had been free of symptoms for more than one year when she contracted influenza in January 1933. Recovery was satisfactory. The rheumatic process remained quiescent. On February 28th she contracted hemolytic streptococcus pharyngitis and was readmitted to the Presbyterian Hospital where she was given 80,000 units of antiserum. The observations during the infection, the symptom-free period and the rheumatic attack are summarized in Table III. The findings are similar to those seen in Table II. Following acute pharyngitis, there occurred a symptom-free interval and then developed the most severe rheumatic attack that this patient has experienced. Polyarthritis was intense.

## RESULTS

The findings in this study of ten patients passively immunized with NY5 antistreptococcus serum are presented together in Table IV. Six individuals developed rheumatic recrudescences and four appeared to escape. Four of the six recrudescences were severe attacks that necessitated bed care for a period of months. In three of these four, the antiserum neutralized in vitro the toxic filtrate of the hemolytic streptococcus associated with the preceding throat infection. Seven of the ten strains were toxin producers, and six of these seven infections were followed by rheumatic recrudescences. In each of the six patients who had recrudescences there was a rise

TABLE III  
Data on Patient M. A., Number 63035

Disease stage	Date	Blood			Throat flora	Clinical observations	Remarks
		W.B.C.	Sedimentation rate	Antistreptolysin titer			
Influenza	1933						
	January 7	7,500		16	Normal	Prostrated, temperature 101°	Severe influenza
	January 8			14		Improving	
	January 30	6,050	12	20	Normal	Symptom-free	No sequelae from influenza
Phase 1 Acute hemolytic streptococcal infection	February 28	14,700	45	25	Hemolytic streptococci predominant	Onset of acute pharyngitis. Temperature 101°	NY5 antiserum 20,000 units
	March 2				Hemolytic streptococci predominant	Acute pharyngitis. Temperature 102°	NY5 antiserum 20,000 units
	March 3				Hemolytic streptococci predominant	Acute pharyngitis. Temperature 102°	NY5 antiserum 20,000 units
	March 4	13,700				Subsiding pharyngitis. Temperature 99°	NY5 antiserum 20,000 units
Phase 2 Symptom-free interval	March 5					Symptom-free	Ekg.—incomplete bundle branch block, present for five years
	March 6	14,700	49			Symptom-free	
	March 7					Symptom-free	
	March 8	11,600	60	111		Symptom-free	
Phase 3 Severe rheumatic attack	March 9				Few hemolytic streptococci		Mild rheumatic symptoms
	March 10						
	March 11						
	March 12						Mild serum sickness
	March 13	12,500	35				
	March 14				Few hemolytic streptococci		
	March 15	14,600	94		Few hemolytic streptococci		
	March 16				Few hemolytic streptococci		
	March 21	10,250	99	125			
	March 23	9,750	91	125			
	March 25	12,050	65				
	March 30	8,920	65				
	April 5						
	April 8						
	April 17	11,100	65	125			
	April 24	8,250	32				
	April 25						
	May 11						
Heart failure	July 25	8,260	12	53		Cardiac insufficiency	Readmitted to Presbyterian Hospital
Good health	October 26			71	Normal	Symptom-free	Living in Brooklyn
	1934 January 22			50	Normal	Symptom-free	Working as salesman

TABLE IV

The effect of passive immunization with streptococcus antitoxin on rheumatic subjects recovering from streptococcus pharyngitis

Patient		Organism				Antiserum		Antistreptolysin titer					Clinical result
Name	Age	Strain of hemolytic streptococcus	Sugar fermentation type	Toxin production	Toxin neutralization with NY5 antiserum	Total number of units	Time of administration	During infection	During quiescent interval	During period of attack			
	years							units per cc.	units per cc.	units per cc.	units per cc.	units per cc.	
M.A.	16	S 65**	Pyogenes	++++	Complete	80,000	During pharyngitis	25		111	125	125	Severe attack 1 week after serum
C.G.	15	S 69	Pyogenes	+++	None	90,000	During pharyngitis	33	33	111	200	250	Severe attack 10 days after serum
F.R.	16	S 67	Subacidus	+++	Complete	100,000	At end of pharyngitis	83		143	250	250	Severe attack 17 days after serum
J.W.	15	S 90	Infrequens	+	Complete	40,000	1 week after pharyngitis	167	167	200	200	250	Severe attack 2 days after serum
J.Z.	30	S 61	Pyogenes	+++	Incomplete	100,000	During pharyngitis	71	83	100	125	100	Mild attack 16 days after serum
M.O.*	21	S 62	Equi	+++	None	80,000	During pharyngitis	25	56		111	167	Mild attack 12 days after serum
W.P.*	21	S 77	Pyogenes	+++	Complete	80,000	At end of pharyngitis	100	111	143	143		Attack apparently escaped
J.R.	15	S 76	Hemolytic II	None		40,000	During pharyngitis	100	56	63	71	63	Attack apparently escaped
P.H.	17	S 100	Pyogenes	None		40,000	1 week after pharyngitis	63	62	71	100		Attack apparently escaped
G.S.	14	S 89	Pyogenes	None		1,000	At end of pharyngitis	33	33	33	33		Attack apparently escaped

\* Developed severe serum sickness the day following the last dose of antitoxin.

\*\* S designates strains from patients treated with serum.

in antistreptolysin titer at the onset of the attack. Of the four individuals who appeared to escape recrudescences, three were infected by organisms that produced no detectable soluble toxin. The antistreptolysin titer remained low in these cases. The results presented in Table IV are in accord with the findings already described (2) for patients who received no antiserum; that is, recrudescences followed infections with agents that produced soluble toxin, and occurred with a response of the antibody mechanism.

#### DISCUSSION

It is seen that active immunization with streptococcus toxin does not afford protection against respiratory infection with hemolytic streptococcus. It is also evident that the introduction of antitoxin just after streptococcus infection is ineffective. This result is strikingly different from the effects of passive immunization following tetanus infection. The observations suggest that if streptococcus toxin plays a rôle in the genesis of rheumatic lesions, the relationship is probably not simply one of direct damage to mesodermal tissues.

One patient, W. P. Number 257703, is of especial interest. While under observation he contracted hemolytic streptococcus pharyngitis in 1931. The organism was a strong toxin producer, and the patient developed a sharp rise in antistreptolysin titer coincident with the onset of a severe rheumatic attack. In the present study, he was also infected by a strain which produced

strong toxin. However, in this instance he developed only a slight rise in antistreptolysin titer and escaped all evidence of rheumatic activity. Whether the occurrence of severe serum disease in this individual modified the antibody response is unknown. His failure to develop a recrudescence suggests that if streptococcus toxin is a factor in the production of rheumatic activity, its effectiveness is dependent upon the immune response of the host.

#### SUMMARY

Active immunization with streptococcus toxin neither prevents streptococcus infection nor inhibits the development of the rheumatic process.

The introduction of protective antibodies just prior to the expected attack does not decrease and may possibly increase the intensity of the rheumatic recrudescence.

The development of rheumatic activity appears to depend not only upon infection with a toxin producing strain of hemolytic streptococcus but also upon the host's immune response to this infection.

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# STUDIES ON THE IMMUNE RESPONSE OF THE RHEUMATIC SUBJECT AND ITS RELATIONSHIP TO ACTIVITY OF THE RHEUMATIC PROCESS. VI. THE SIGNIFICANCE OF THE RISE OF ANTISTREPTOLYSIN LEVEL IN THE DEVELOPMENT OF RHEUMATIC ACTIVITY<sup>1</sup>

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The authors have observed that respiratory infection with a strain of hemolytic streptococcus producing strong toxin is not invariably followed by rheumatic recrudescence<sup>2</sup> in a susceptible subject. This observation has been made under several different circumstances. (a) Two rheumatic children escaped rheumatic activity in spite of infection with a strain of hemolytic streptococcus that produced severe recrudescences in fourteen others living in the same home (1). (b) Infections with a scarlatinal strain of hemolytic streptococcus, occurring simultaneously in two rheumatic siblings were followed by rheumatic attacks, one of which was severe and prolonged, the other extremely mild (2). (c) Scarlet fever in one rheumatic subject under close observation was followed by only mild carditis. (d) Several of the strains described in the fourth paper of this series (2) produced strong toxin and streptolysin but were not effective in initiating recrudescences. The one characteristic common to all of these individuals who escaped attacks was the failure to develop a significant rise in antistreptolysin titer. The present paper deals with the significance of changes in antistreptolysin level in the rheumatic subject.

## *The natural antistreptolysin level and the average range in good health*

It has already been shown (3) that the natural antistreptolysin level in subjects who have been free of hemolytic streptococcus infection is ap-

proximately 50 units. The range of titers to be expected in apparently healthy persons living in New York City was determined in a study of 146 individuals of various ages. They are reported in five groups as specified in Table I

TABLE I  
*The frequency of high and low antistreptolysin titers among several groups of subjects living in New York City*

Group	Number of cases with titer of		
	100 units or less	Between 100 and 200 units	200 units or more
Mothers.....	29	7	1
Babies.....	25	10	2
Medical students.....	8	1	1
Nurses—probationers (1931).....	14	5	3
Nurses—probationers (1932).....	34	6	0
Total.....	110	29	7
Per cent.....	75	20	5

These data are summarized in Table III where it is seen that the median titer for 176 individuals in good health was 83 units. This is significantly higher than the natural level of 50 units.

These findings serve as an index of what may be expected in New York City in the population as a whole. The results show that 75 per cent had antistreptolysin titers of 100 units or less, 20 per cent had titers of 200 units or more, 5 per cent were intermediate. The median<sup>3</sup> of the 146

<sup>1</sup> The work reported in this communication was carried out under The W. K. Kellogg Foundation.

<sup>2</sup> The term "recrudescence" is applied to the development of an acute attack in a known rheumatic subject, following a period of quiescence. It is not to be confused with an exacerbation of symptoms, such as may occur late in a polycyclic rheumatic attack.

<sup>3</sup> To facilitate comparisons between groups, medians have been used throughout rather than averages, as it is not mathematically permissible to average quantities of such different magnitudes as the titers in question.



determinations was 71 units, slightly higher than the natural level of 50 units. This indicates the difference between the range of titers to be expected during "good health" or following other infections and the natural antistreptolysin level of individuals who have been free of hemolytic streptococcus disease for a long period of time.

*The range of antistreptolysin titers following hemolytic streptococcus infection*

The authors are in entire agreement with Todd (4) that the presence of an antistreptolysin titer of 200 units or more is strong evidence of recent infection with hemolytic streptococcus. However, in interpreting the significance of titers below 200 units it is important to know the frequency of such levels in persons convalescing from these infections. For this purpose, determinations were made on sera obtained from a number of suitable patients. The readings were of two kinds, single determinations made four weeks after recovery from infection, and determinations made serially for six months following infection.

Single observations in a group of 26 cases showed that 10 per cent (following scarlet fever), 16 per cent (following mastoiditis) and 50 per cent (following pharyngitis) had titers below 200 units. In 24 patients with hemolytic streptococcus pharyngitis whose titers were determined repeatedly, 55 per cent developed maximum levels of less than 200 units. Most of these low titers followed infections contracted during the fall and winter months.<sup>4</sup> There was wide variation in the type of titer curve developed. Some individuals (Type A) showed a progressive increase in antistreptolysin level, which reached a peak in five to twenty weeks. Other individuals (Type B) had a sharp initial rise, which was maintained for only a few weeks. Nearly all of the patients developed a secondary rise of titer some months after the primary maximum. Illustrative curves of both types are presented in Figure 1. The

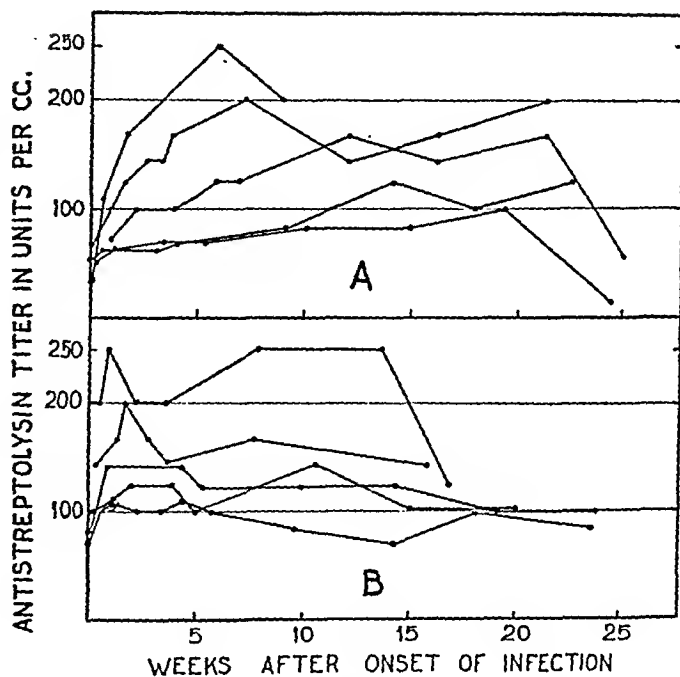


FIG. 1. THE DEVELOPMENT OF ANTISTREPTOLYSIN TITERS IN YOUNG ADULT, NON-RHEUMATIC SUBJECTS FOLLOWING THROAT INFECTIONS WITH HEMOLYTIC STREPTOCOCCUS.

data for all the single and serial determinations are presented later, along with the observations on rheumatic patients in Table VII.

*Antistreptolysin titers during the attack of acute rheumatism*

Working in conjunction with Dr. E. W. Todd, the authors reported finding high antistreptolysin titers in the sera of patients with acute rheumatism (5). Similar studies published by Myers and Keefer (6) of Boston have substantiated these findings in part, but have raised certain questions which it seems advisable to discuss at this point, before presenting any more data. Myers and Keefer agree that the antistreptolysin titer in acute rheumatism is elevated to a level equal to that following proven streptococcal infections; but they question any significance being attached to readings of 200 or 250 units, since 200 was the "average" level of their normal control group. The choice of a few hospital assistants for their "normal control group," may well account for these high readings. The failure of Wilson *et al.* (7) to detect the significance of the antistreptolysin titer in children with rheumatic fever may result from their use of averages, ap-

<sup>4</sup> This seasonal variation is mentioned because the authors have also noted a similar fluctuation in production of toxin and in the frequency with which these infections are followed by a rheumatic attack. In New York City the activity of hemolytic streptococcus appears to be at a minimum in the early autumn, and to increase progressively throughout the winter and spring months.

plied at a critical point in their argument to only ten cases. Since titer readings increase geometrically, arithmetic averages are meaningless.

Antistreptolysin determinations have been made in this laboratory on all of the authors' patients with frank attacks of rheumatic fever, those with chorea being excluded. Altogether, the sera of 175 patients were examined in 1933, 1934 and 1935. Single readings were made on the first group of patients; weekly titrations were made in 1934 and 1935. The results were as follows: In 1933, the median was 250 units, the (geometric) mean 224; in 1934, the median was 500 units, the mean 430 units; in 1935, the median was 500 units, the mean 562. These data are presented in Table VII.

The occurrence of these high titers in acute rheumatism did not appear to depend on the age of the individual. The authors' patients ranged from 18 months to 50 years of age.<sup>5</sup> The percentage distribution of titer levels in relation to age is shown in Table II.

TABLE II

*Percentage distribution of antistreptolysin titers in relation to age, in 271 patients with acute rheumatism 1930-1935*

Age groups	Number of cases	Range of antistreptolysin titers						Median titers
		50 to 83	100 to 167	200 to 333	500 to 833	1000 to 1667	2500	
<i>years</i>								
0 to 2	5			20	60	20		500
3 to 7	22			35	30	30	5	500
8 to 13	115	1	4	48	24	19	4	333
14 to 21	72	1	10	48	30	10	1	333
22 to 30	29		21	59	10	10		250
31 to 50	28		30	50	17		3	250

It is seen in Table II that the titer distributions were approximately the same at all ages. There was a slight tendency towards higher levels in the younger age groups and a similar tendency towards lower levels in adults with acute rheumatism.

<sup>5</sup> In 80 per cent of 37 cases studied, blood from the cord of the newborn contained more antistreptolysin than the corresponding maternal blood.

### *The fall in antistreptolysin titer during subsidence of rheumatic activity*

It was possible to observe 41 of these patients for two years following an attack of acute rheumatism and to obtain sera for titer determination at appropriate intervals. So far as could be determined, none of these patients was reinfecting during this period. The changes in titer level during convalescence are presented in Table III.

The changes in titer seen in Table III are similar to the observations made at The Pelham Home (1). The rate of fall in titer during convalescence varied markedly. In no instance was the titer higher during recovery than during the acute attack. Sera of all individuals tested at the end of six months showed a fall in titer, with the exception of one child who maintained a titer of 333 units for two years. More than half of the patients had reached natural levels at the end of two years; the remainder had only a slight elevation of titer. The median value for the group at this time was 71 units. In contrast, the median value for the same patients during the acute attack was 250 units. From these observations it is seen that the antistreptolysin titer diminishes as the rheumatic process becomes quiescent. This is believed to indicate subsiding activity of the tissues producing antibody to hemolytic streptococcus.

In summary it is apparent that the range of antistreptolysin titer during the attack of acute rheumatism is (1) similar to that observed during convalescence from scarlet fever, (2) higher than that following hemolytic streptococcus pharyngitis, (3) considerably higher than that of rheumatic subjects apparently quiescent, (4) strikingly higher than that of individuals in good health or with diseases not of hemolytic streptococcus origin.

Serial antistreptolysin determinations make it possible to investigate the time relationship between the development of rheumatic activity and the production of antibodies to hemolytic streptococcus. The authors are of the opinion that although the antistreptolysin titer represents only a fraction of this antibody response, it is the best available index of the total immune response to infection with this agent. The following observations are presented in detail to show a relation-

TABLE III

*Antistreptolysin titers of patients recovering from acute rheumatism (units per cc.)*

Name	Acute rheumatism				Symptom free							Apparently quiescent													
	(Month) 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	
Falman.....	250								143																
Neal.....	250	250									111	100													
Pagano.....	333											143		100											
Paul.....	333											100													
Riccardi.....	333	333		333						100		100													
Romorofsky.....	200																							71	
Scibelli.....	250											200				143						125		111	
Sullivan, M.....	1000											167													
Sullivan, H.....	333							250																	
Anderson.....		500					143												100						
Acconero, P.....	83	125									83														
Braun.....	333						200		143			143												50	
Clifford.....	167						50					50												125	
Clowry.....	500										125														
deMario.....	250		250									125													
DeBiasi.....		250																	111						
Feeley.....	333				167		200					100												100	
Franklin.....	250					167						125												33	
Giralt.....	500		500				50					50								33					
Hall.....	250		100		50							50												71	
Herman.....					200							143												125	
Hickey.....					167				167															83	
Howe.....	500				250				71																
Korson.....			111									50												25	
Kouba.....	500					125	125	111								71								50	
Lally.....	250																							100	
Lun.....	333																								
McDonald.....	333		500												250									50	
Mackay.....				111																				143	
Maurer.....	1250	1250																							
Mazorra.....	143			71																				333	
Mazzia.....	250	333								333		333													
Mullins.....	500	500														111									
Oxford.....	167					111																			
Prek.....	333							83		83															
Rexach.....	125					71						33													
Shanahan.....	500						167			200															
Stone.....	250	250	250						200			167													
Temestocle.....	333		333									200													
Tsea.....	250													111											
Gallano.....	250												100												

ship between rheumatic fever and activity of the antibody-producing mechanism.

*The relationship between the height of the antistreptolysin titer attained and the severity of the rheumatic attack*

The authors have studied a group of rheumatic subjects over a period of years to determine the effect of respiratory infections on their disease. The members of this group who developed acute rheumatism showed a coincident rise in antistreptolysin titer with each recrudescence. Furthermore, those rheumatic subjects who appeared to escape activity of the rheumatic process following respiratory infection usually showed little or no change in titer level. Five year observations on two illustrative patients are presented along with their antistreptolysin titer curves. Each of these patients, one child and one young adult, had several respiratory infections with different types of hemolytic streptococcus in the course of the observation period.

CASE HISTORIES

*H. D., Number 69558.* The patient, an American boy of Italian parentage, has been under the authors' care since 1928 when he was first seen at the age of seven with rheumatic heart disease. Between 1930 and 1935, he was kept under close clinical observation. Throat cultures were examined each month and blood samples obtained at intervals for antistreptolysin determinations. Two throat infections occurred during the five year period.

The first of these hemolytic streptococcus infections was contracted on February 17, 1934. The patient was admitted to Babies Hospital where he ran the course of acute pharyngitis with a three day fever. On March 1, the sedimentation rate of the blood, which had been falling, showed a moderate rise. He was discharged on the tenth of the month, symptom-free, with, however, an elevated sedimentation rate. Three weeks later he developed a frank attack of polyarthritis, accompanied by marked increase in the sedimentation rate. This attack required hospitalization and prolonged convalescent care.

The patient was again in excellent condition when he contracted the next throat infection, March 9, 1935. He was cared for in the wards of the Presbyterian Hospital. Recovery from acute pharyngitis was rapid and convalescence uneventful until April 2 when the pulse rate, sedimentation rate of the blood and white blood count

rose slightly and electrocardiographic changes appeared—"T waves have become definitely abnormal suggesting heart muscle damage, possibly active." All of these laboratory changes disappeared within a few days, and the patient was in good condition until April 23 when he developed epistaxis, then polyarthrititis, followed on May 1 by a severe rheumatic attack with pericarditis.

Hemolytic streptococcus was recovered from the patient's throat once in 1931 and 1932, but did not appear to cause local infections or initiate rheumatic activity. On February 16, 1934, hemolytic streptococcus appeared in predominance during the period of pharyngitis and persisted until January 1935. On March 9, 1935, the organ-

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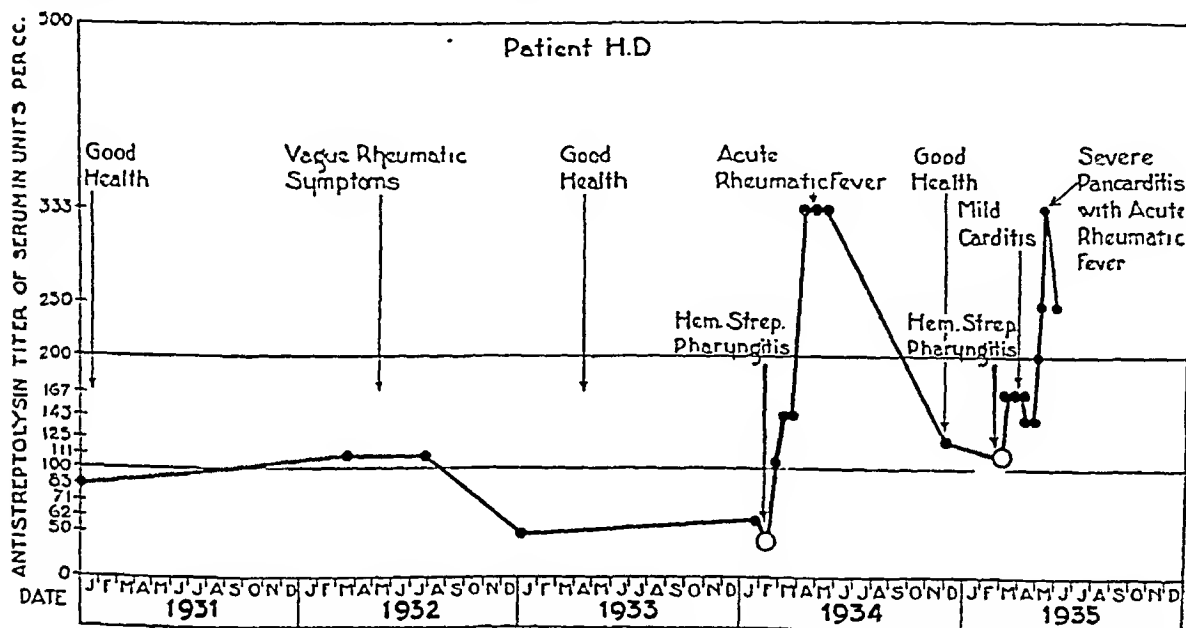


FIG. 2. CHANGES IN ANTISTREPTOLYSIN LEVEL IN PATIENT H. D., FOLLOWING HEMOLYTIC STREPTOCOCCUS INFECTIONS FROM 1931 TO 1935

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*M. O., Number 82235.* The second patient, an Irish maid of eighteen, came under the authors' observation in May 1929 when she was admitted to the Presbyterian Hospital with acute rheumatic fever and mitral stenosis. At that time her throat flora contained hemolytic streptococcus. Between 1929 and 1935 she reported monthly for examination and throat cultures. Samples of blood for antistreptolysin determination were obtained at appropriate intervals. During five years of observation she contracted four distinct throat infections.

On February 21, 1931, she contracted acute pharyngitis with fever for three days. Muscle pains, mild pyrexia and tachycardia appeared on March 7, 1931. The electrocardiographic tracing showed extensive migration of the pacemaker. All symptoms and cardiac changes disappeared in one week. On January 13, 1932, she again contracted acute pharyngitis with a three day fever. During the latter part of February vague manifestations were noted. Suddenly, on March 7, 1932, she became

TABLE III

*Antistreptolysin titers of patients recovering from acute rheumatism (units per cc.)*

Name	Acute rheumatism				Symptom free							Apparently quiescent													
	(Month) 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	
Faiman.....	250								143																
Neal.....	250	250																							
Pagano.....	333										111														
Paul.....	333											100													
Riccardi.....	333	333		333						100		143		100											
Romorofsky.....	200											100													
Scibelli.....	250																								
Sullivan, M.....	1000											200				143						125			71
Sullivan, H.....	333							250				167													111
Anderson.....		500																							
Acconero, P.....	83	125					143													100					
Braun.....	333						200			143															
Clifford.....	167						50																		50
Clowry.....	500																								125
deMario.....	250			250								125													
DeBiasi.....		250																							
Feeley.....	333					167		200													111				100
Franklin.....	250						167																		
Giralt.....	500			500				50																	33
Hall.....	250			100		50															33				71
Herman.....						200																			125
Hickey.....						167				167															83
Howe.....	500					250				71															
Korson.....			111																						25
Kouba.....	500						125	125	111																
Lally.....	250																								50
Lun.....	333																								100
McDonald.....	333			500																					
Mackay.....					111																				50
Maurer.....	1250	1250																							143
Mazorra.....	143				71																				
Mazzia.....	250	333																							333
Mullins.....	500	500																							
Oxford.....	167						111										111								
Prek.....	333								83			83													
Rexach.....	125						71																		
Shanahan.....	500							167				200													
Stone.....	250	250	250							200															
Temestocle.....	333			333																					
Tsca.....	250																								
Galiano.....	250													100			111								

ship between rheumatic fever and activity of the antibody-producing mechanism.

*The relationship between the height of the antistreptolysin titer attained and the severity of the rheumatic attack*

The authors have studied a group of rheumatic subjects over a period of years to determine the effect of respiratory infections on their disease. The members of this group who developed acute rheumatism showed a coincident rise in antistreptolysin titer with each recrudescence. Furthermore, those rheumatic subjects who appeared to escape activity of the rheumatic process following respiratory infection usually showed little or no change in titer level. Five year observations on two illustrative patients are presented along with their antistreptolysin titer curves. Each of these patients, one child and one young adult, had several respiratory infections with different types of hemolytic streptococcus in the course of the observation period.

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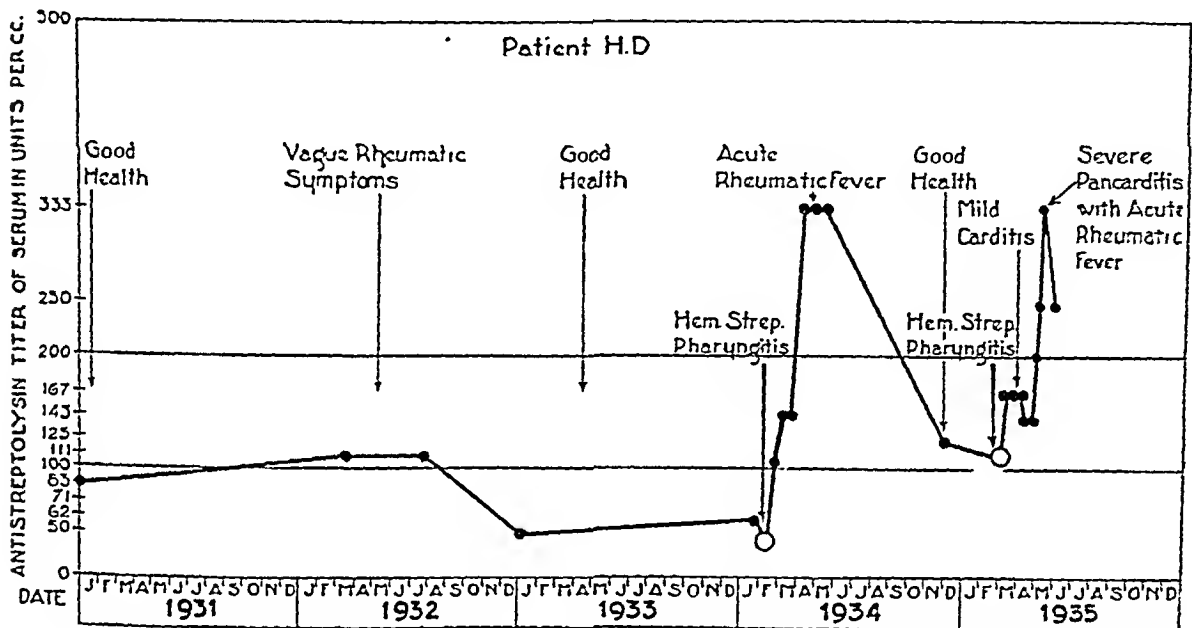


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acutely ill with severe polyarthritides, pyrexia, dyspnea and tachycardia. The electrocardiographic tracing again showed nodal rhythm with shifting pacemaker, and there was a return to normal in one week. Symptomatic recovery from this attack occurred in three weeks. On January 30, 1933, she again contracted acute pharyngitis. This infection was severe and was followed by extensive cervical adenitis. While at rest in bed in the Presbyterian Hospital on February 17, 1933, she developed muscle pains, epistaxis, mild pyrexia, leukocytosis and increased sedimentation rate of the blood without electrocardiographic changes. All evidences of rheumatic activity disappeared in five days. On December 29, 1934, she again contracted acute pharyngitis with a three day fever. This infection was followed by three months of excellent health without symptoms, signs or any laboratory evidence suggesting rheumatic activity. Another infection in April, 1935, was followed by a mild rheumatic attack.

The throat cultures from this patient showed the arrival of hemolytic streptococcus on February 21, 1931; on January 13, 1932; on January 30, 1933, and on December 29, 1934. This organism was predominant or present in large quantities in both tonsillar fossae during

was moderately elevated to 125 units. After the patient had been in good health for eight months the titer fell to the natural level of 50 units. There was no change in titer during the 1932 pharyngitis; however, with the onset of acute rheumatism on March 10, 1932, the level rose to 333 units. This was followed by a gradual decline in antistreptolysin level. During the severe throat infection in 1933 the titer reached a value subnormal for this patient, then one week later returned to natural level, and with the onset of mild rheumatic symptoms rose to 111 units and then to 166 units. The patient maintained a constant level of 71 units through 1934. Following pharyngitis in December, 1934, the titer remained practically stationary, and the patient symptom-free. However, another throat infection in April, 1935, was followed by a rise in titer to 143 units, coincident with the development of rheumatic symptoms.

In summary, there was a close correlation between the degree of rheumatic activity and the height to which the antistreptolysin titer rose. In the absence of appreciable change in titer, this rheumatic subject infected with hemolytic streptococcus escaped all clinical and laboratory signs of recrudescence. The changes in antistreptolysin level are presented in Figure 3.

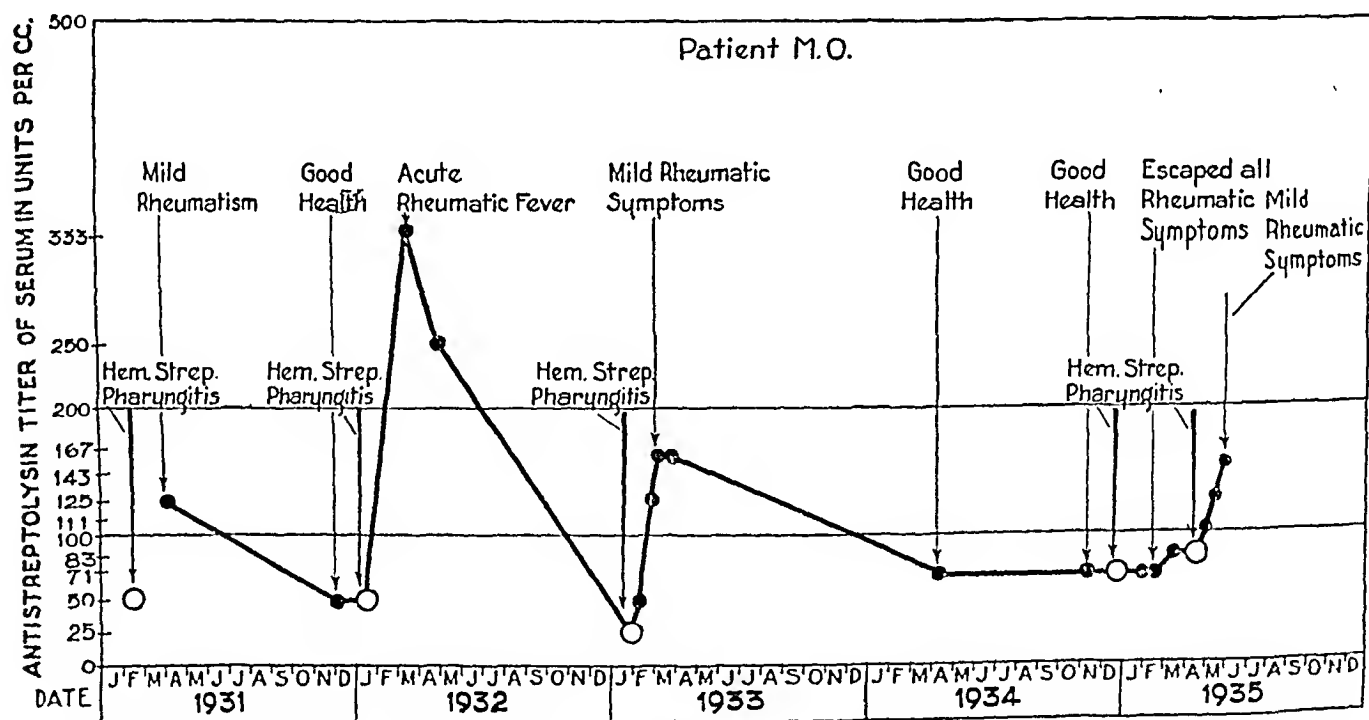


FIG. 3. CHANGES IN ANTISTREPTOLYSIN LEVEL IN PATIENT M. O., FOLLOWING HEMOLYTIC STREPTOCOCCUS INFECTIONS FROM 1931 TO 1935

each of these infections and disappeared entirely from the pharyngeal mucosa in one to six weeks with all but the last infection. Studies of the cultural and biological characteristics of these organisms indicated that these infections were caused by different types of beta hemolytic streptococcus.

The antistreptolysin studies were begun during the mild rheumatic attack in 1931. At this time the level

The observations on the two patients just discussed suggest a relationship between the height of antistreptolysin level attained and the intensity of the rheumatic attack. In order to investigate this possibility in a large number of subjects with acute rheumatism, the authors have made serial determinations on 72 consecutive admissions to





The classes in Table IV have been defined as follows:

- Class I*—Fulminating polycyclic attack with intense pancarditis.  
*Class II*—Continuous, severe carditis, insidious in onset.  
*Class III*—Severe monocyclic polyarthrititis with mild carditis.  
*Class IV*—Severe polycyclic polyarthrititis with mild carditis.  
*Class V*—Mild, vague rheumatic attacks of short duration.  
*Class VI*—Mixed types.

*Class I.* Most of the patients who survived for three weeks or more developed extremely high antistreptolysin titers. Two died during the first week of the attack probably before the titer levels had reached maximum values. A similar observation was made during The Pelham Home epidemic (1). Illustrative titer curves are presented in Figure 4.

*Class II.* Most of these individuals came under observation late in the rheumatic attack and, although symptom-free, developed rapidly progressive carditis while at rest in bed. All of these children developed extremely high antistreptolysin levels, as shown in sample curves, Figure 4.

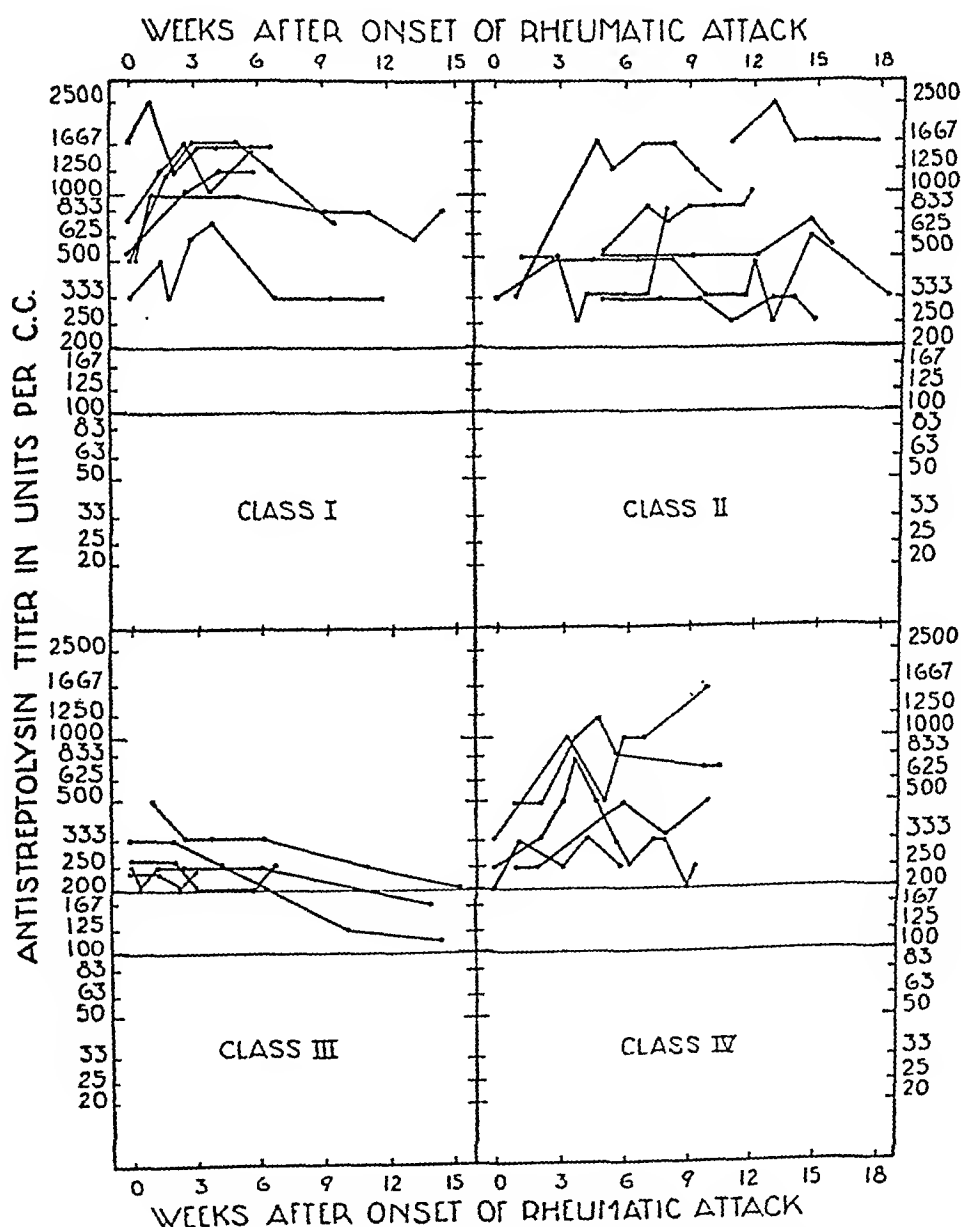


FIG. 4. ANTISTREPTOLYSIN TITER CURVES OF FOUR CLINICAL TYPES OF PATIENTS WITH RHEUMATIC FEVER

*Class III.* These patients had severe but brief rheumatic attacks without serious carditis. The disease process seemed to subside rapidly and spontaneously. The attacks were limited to a single cycle of approximately two weeks duration. None of the titers exceeded 500 units at any time and all tended to fall shortly after the disappearance of symptoms. Illustrative curves are shown in Figure 4.

*Class IV.* In contrast, these patients who at first were clinically similar to those in Class III, developed repeated cycles of polyarthritis. These exacerbations were accompanied by marked increases in titer levels, bringing them into the titer range of Classes I and II. See Figure 4.

*Class V.* These patients had vague manifestations with laboratory findings indicating mild rheumatic activity. Recovery was prompt and in most instances the titer levels were only slightly elevated.

*Class VI.* These individuals presented a mixed clinical picture. It has been considered advisable to exclude them from the well defined groups.

In summary, it appears that extremely high

antistreptolysin levels (over 500 units) are associated with intense or prolonged rheumatic activity. The lower levels seem to be associated with acute rheumatism that is either short in duration or mild in character. These observations are believed to indicate that the intensity of the rheumatic attack is associated with the degree of activity of the subject's antibody production to hemolytic streptococcus.

*The relationship between the rise in antistreptolysin titer and the onset of rheumatic activity*

Another relationship suggested in the titer curves in Figures 2 and 3 is the coincidence of a sharp rise in titer with the onset of the rheumatic attack. This observation was found to be true in The Pelham Home epidemic (1). In order to study this time relationship in a significant number of cases, the authors made determinations on 30 patients during hemolytic streptococcus pharyngitis, the quiescent interval and the attack of acute rheumatism. The determinations for the entire group are presented in Table V.

TABLE V

*The development of antistreptolysin titer (units per cc.) between hemolytic streptococcus infection and the rheumatic attack*

Patient	Before infection	During infection	Quiescent interval (weeks)							Acute rheumatism (months)												Character of attack
			1	2	3	4	5	6	7	1	2	3	4	5	6	7	8	9	10	11	12	
Aceto.....	200					R *				500	1000											Severe
Aita.....	20	25	25	111 R						125	125	125			500				500		50	Severe
Alfisi, A.....		25		50						200	167	167									33	Mild
Alfisi, S.....	125				200 R					250	333										125	Mild
Bent.....	167	250	500		R					1667	2500	1667										Severe
Cerney.....	50	25	71				R			1250							143					Severe
D'Amico.....	71	50	100	143			143		143 R	333	500	333					125					Moderate
D'Amico (2d atk)...	125	111	167	167	167		143		143 R	333	250											Moderate
Digerlando.....		83		167		R				250	250											Mild
Earls.....	250		333	333						500												Moderate
Einhorn.....	63	50	125	125						500	556		333									Moderate
Frizzell.....		250	167	167	167	167			R	250	250						250					Mild
Gilligan.....	33	33	33	111 R						200	500						125		71		71	Moderate
Hanke.....		50			R					200												Moderate
Hickey.....	83	125	200		R					200	1000	833	625									Severe
Lemone.....	143	125	166		R					250	500	500					200					Moderate
Maurer.....	143		250 R							500	2500						333		333			Severe
Murphy.....		200								333					333							Mild
Neal.....	100									500	1000											Moderate
Neal (2d atk).....		250								1000	833						250					Moderate
O'Hare.....	50	50								333	250											Moderate
O'Hare.....	83	100		125		R				143										25		Moderate
Pagano.....	100		143 R							200	2500	1667										Mild
Prek.....	50									143	333	250	250	167	143		1250			200		Severe
Riccardi.....	100									250	250	250	250									Moderate
Rifkin.....	100	100	143 R							333	250	167	143			250						Moderate
Schlosser.....		83			250 R					200									111			Moderate
Schibelli.....	71		71 R							250	250	250							50			Moderate
Servetman.....	33		125 R		167 R					250												Severe
Terentino.....			167	1250 R						500		500		250		200						Severe
Weber.....			167	167 R						200	250								143			Severe
Wynne.....	50	25	25	33	33	125 R				250		250			111					100		Mild

\* R indicates time of onset of rheumatic symptoms.

It is seen in Table V that the onset of each rheumatic attack occurred at the time that the antistreptolysin titer was rising. The development of the rheumatic attack coincident with the immune response to hemolytic streptococcus has been a consistent finding during these studies.

*The maintenance of low antistreptolysin level in rheumatic subjects who escaped recrudescence following hemolytic streptococcus pharyngitis*

The authors have pointed out (5) that when rheumatic subjects are infected with hemolytic streptococcus, some develop frank recrudescences; others get mild rheumatic symptoms and the rest appear to escape rheumatic activity entirely. The present section concerns itself with the last of these groups of patients. Their titer changes are presented in Table VI.

lar observations are to be found in Figure 2, in an earlier paper of this series (2), and were also made in the epidemic at The Pelham Home (1). Representative titer curves of patients who developed attacks and of those who did not are shown in Figure 5.

All of the titers discussed in this study have been classified in Table VII with reference to their frequency distribution. The logarithms of the units have been used as a basis for classification, (a) to correct for the skewness of the distribution of titer units on an arithmetic scale, and (b) thus permit the calculation<sup>a</sup> of means and probable errors, and the determination by statistical methods of the significance of differences between groups. As already mentioned, the titers of the first five groups are taken from single determinations and those in the last seven groups represent the maximum of a series of determinations.

TABLE VI

*Antistreptolysin titers (units per cc.) in individuals with streptococcus pharyngitis not followed by rheumatic attacks*

Name	Pharyngitis		After pharyngitis																		Months	
	Before	During	Weeks																		6	12
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18		
Auris.....		50	50		50																	
Bourie.....	143	125	167	167	167	200	167	167							143							
Brogan.....		71	143	167																		
Byrnes.....	71	50	50	50				50		250												50
Delaney.....			71		71	100																
Elphick.....		333	200		200				333						333							
Fromer.....	250		250	250	250		250														333	
Grassi.....		167	250	250	250	250	250	250				250				250						
Grasso.....		333	333				333						333									
Jackson.....		83	71	71	71				71										71			
Levy.....			63			63			71				71									
McFarland.....		143	143	143	143	143						143							125	143		
Miller, H.....	125	143						111														
Miller, O.....	125	100		111	111	111	111													125	125	
Morris.....		12 *	71	167	125																	
Murphy.....		83	83		100	143																
O'Brien.....		111				125	111			111									100			
O'Hare.....	71	71	71			71							83									50
Phillips.....	50	71	50	71	50	71	71															
Pisello.....			125	125				143	143				143									
Rappa.....	71	71	71	83	111																	
Rodriquez.....			50	50															50			
Skea.....		250			200			143	143				125	125								71
Solomon.....		33	33	33	33																	
Vitale.....	167	167	167		143					125												

\* This infection was severe and probably accounts for the depressed titer level.

It is seen from Table VI that in individuals contracting pharyngitis with hemolytic streptococcus not followed by rheumatic recrudescence, the antistreptolysin titer either fell, remained stationary or rose slightly. These titer levels are in distinct contrast to those of patients who developed frank attacks of acute rheumatism. Simi-

<sup>a</sup> The statistical methods used in this paper were taken from Garrett, H. E., Statistics in Psychology and Education. New York, Longmans, Green and Company, 1926. The calculation of those probable errors which depend upon relatively few cases (13 or less) was made by the formulae of R. A. Fisher, as given by Dunn, H. L., Physiol. Reviews, 1929, 9, 275.

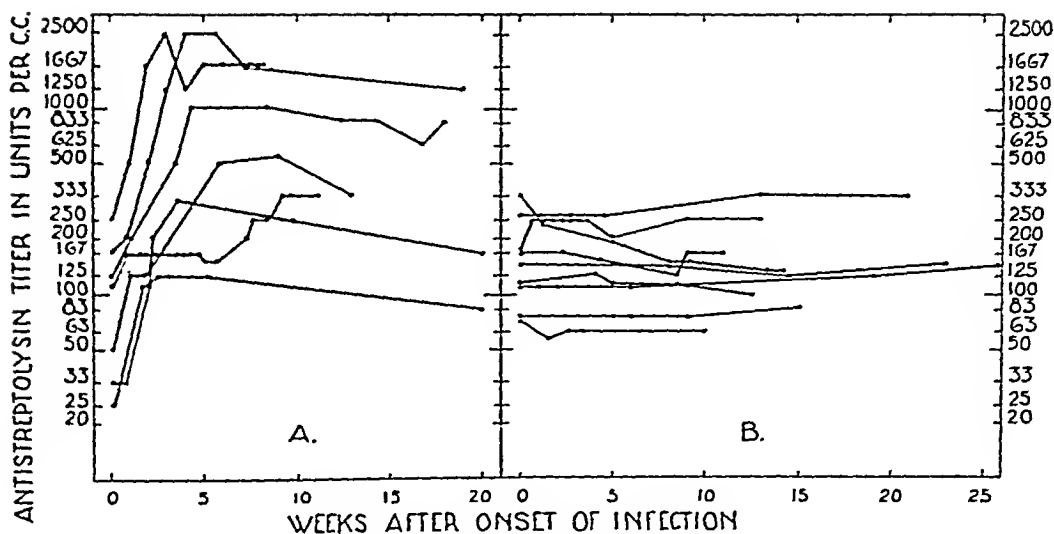


FIG. 5. ANTISTREPTOLYSIN TITER CURVES OF RHEUMATIC SUBJECTS DURING AND AFTER HEMOLYTIC STREPTOCOCCUS PHARYNGITIS

A. Developed rheumatic attacks.  
B. Escaped rheumatic attacks.

As is seen in Table VII the geometric means (calculated from the average log  $u$ ) differ only slightly from the medians. This indicates that the logs of the titers have an approximately normal distribution. In view of the probable errors of the average log  $u$ 's, the following conclusions can be drawn: (a) There is no significant difference between Group 1 and 2; (b) Groups 3, 4, and 5 are significantly higher than both 1 and 2; (c) There is an apparent difference between Groups 4 and 5; however, because of the small number of cases in Group 4, it is unwise to draw conclusions; (d) Groups 6 and 3 are almost identical; (e) Group 7 is significantly lower than 6 and 8; (f) The average mean log  $u$  of Groups 7 and 8 does not differ significantly from the mean log  $u$  of Group 6; (g) There is no significant difference between Groups 8, 9 and 10; (h) The differences between these three groups and Group 5, which are significant, may be attributed to the method of collecting material; (i) Groups 11 and 12 are significantly lower than Groups 8, 9 and 10.

From the data obtained in 1934 and 1935, the range of titers to be expected with rheumatic fever in terms of log units is  $2.69 \pm 0.31$  (S.D.). This will include  $\frac{2}{3}$  of such cases, and the range of  $2.69 \pm (3 \times 0.31)$  will include practically all patients with acute rheumatism. Expressed in units this range corresponds to titers of 240 to

1260 units for  $\frac{2}{3}$  of the cases with the largest number falling at about 490 units.<sup>7</sup>

The patients who escaped recrudescence following pharyngitis with hemolytic streptococcus (Group 7) showed distinctly less antistreptolysin response to infection than either non-rheumatic subjects following comparable infection or patients with acute rheumatism. This cannot be attributed in all cases to the nature of the infecting agent (2) but in some instances seems to be associated with a diminished immune response of the host.

#### DISCUSSION

Sufficient data are not yet available to determine whether the antibody response of the rheumatic subject differs from that of the non-rheumatic subject. This discussion will therefore be confined to rheumatic subjects. The data presented for this group show wide variations in the antibody response to hemolytic streptococcus infection. These variations seem significant in determining the development of rheumatic activity. In general, the greater the antibody response, the more severe the accompanying rheumatic attack; and conversely, in the absence of antibody response the rheumatic attack fails to develop. The

<sup>7</sup> The authors are indebted to Dr. A. A. Weech for advice in applying statistical methods to these data.

TABLE VII—The distribution of antistreptolysin titers in various groups of subjects

Section	Group	Num-ber of cases	Approximate log of units Titer classes in terms of units	1.0	1.2	1.4	1.6	1.8	2.0	2.2	2.4	2.6	2.8	3.0	3.2	3.4	Median (units)	Geo-metric mean	Mean log #	Prob-able error	Standard deviation
				10	16, 20	25	33, 50, 63, 71	83, 100, 111, 125	125, 143, 167, 200	200, 250	333, 500	556, 625, 714	833, 1000, 1250	1250, 1667	2500						
A—Single determinations	1	176	Good health	1	6	13	26	38	54	24	8	4					83	74	1.87	.015	.30
	2	21	Ward patients with other diseases	1		1	4	6	7	2							63	65	1.81	.039	.27
	3	10	After hemolytic streptococcus pharyngitis, non-rheumatic subjects						1	5	2	2					183	197	2.30	.031	.14
	4	10	After scarlatina, non-rheumatic subjects							1	2	4	2		1		333	347	2.54	.064	.29
	5	39	Acute rheumatic fever, 1933							7	9	9	7	6	1		250	224	2.35	.025	.23
B—Serial determinations	6	25	Non-rheumatic sub-jects							6	9	7	1	2			183	191	2.28	.024	.18
	7	25	After hemolytic strep-tococcus pharyngitis				4	3	5	7	4	2					134	122	2.09	.036	.27
	8	30	Rheumatic subjects without attack						1	4	6	7	4	4	1	3	333	440	2.64	.044	.36
	9	66	Rheumatic subjects with attack						1	6	17	18	11	8	3	2	500	430	2.63	.025	.30
	10	72	Acute rheumatic fever 1934								3	10	24	12	11	4	500	562	2.75	.025	.31
	11	30	Acute rheumatic fever 1935								10	1	1				125	114	2.06	.028	.23
			3 to 12 months symptom free				5	2	11												
	12	23	1 to 2 years			1	4	3	12	1	1	1					105	90	1.96	.034	.25

titer level attained by any individual patient seems to be the result of at least three factors: the character of the infecting strain, the intensity of the patient's response to that particular infection, and the capacity for antibody production characteristic of the individual. The close time relationship between the antistreptolysin rise and the onset of rheumatic activity, together with the close correspondence between the absence of such rise in titer and the quiescence of the rheumatic process, has led to the belief that the association is not accidental. However, the mechanism responsible for the simultaneous occurrence of these immunological and clinical changes remains to be determined. One of a number of possible explanations is dealt with in the following paper (8).

SUMMARY

The median of the antistreptolysin determinations on 176 individuals in good health was 71 units. This is somewhat higher than the natural human level of 50 units.

The median titer developed in acute rheumatism was 500 units, and the geometric mean 490 units. In most instances the titer returned to approximately natural level within a period of one year.

The onset of acute rheumatism coincided with a sharp rise in antistreptolysin titer.

Rheumatic patients infected with hemolytic streptococcus who escaped recrudescence, showed little or no change in antistreptolysin titer.

The relation of the immune response of the host to the development of rheumatic activity is discussed.

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TABLE II  
The reactions of a group of 20 rheumatic subjects to splenectomy

Group	Name	Preoperative findings				Postoperative findings		
		Recent hemolytic streptococcus infection	Clinical state	Throat flora	W.B.C.	W.B.C.	Anti-streptolysin titer *	Symptoms
A.	J.H.	None recognized	Apparently quiescent	Normal	5,900	10,050	100	None
	C.T.	None recognized	Apparently quiescent	Normal	6,650	9,600	250	None
	V.O.	None recognized	Apparently quiescent	Normal	6,400	12,150	50	None
	T.M.	None recognized	Apparently quiescent	Normal	7,700	10,000	100	None
	D.M.	None recognized	Apparently quiescent	Normal	8,150	6,850	33	None
	N.F.	None recognized	Apparently quiescent	Normal	7,500	9,000	200	None
	M.G.	None recognized	Apparently quiescent	Normal	8,100	8,100	250	None
	D.H.	None recognized	Apparently quiescent	Normal	7,500	9,250	100	None
	E.B.	None recognized	Apparently quiescent	Normal	7,000	8,200	50	None
	D.D.	None recognized	Apparently quiescent	Normal	6,500	16,850	50	None
	D.G.	None recognized	Apparently quiescent	Normal	9,400	14,000	50	None
B.	E.C.	Pharyngitis 4 months previously	Persistent mild carditis	Normal	6,800	13,300	143	Ekg. T waves became inverted post-operative
	M.K.	Pharyngitis 2 months previously	Apparently quiescent	Normal	5,500	7,250	167	Ekg. minor changes in T waves post-operative
	S.G.	Pharyngitis 1 month previously	Apparently quiescent	Normal	8,850	11,950	167	Mild pyrexia temperature 100 to 102°.
	G.H.	Pharyngitis 6 months previously	Apparently quiescent	Normal	10,750	18,450	167	Inversion of T waves postoperative
C.	D.K.	Pharyngitis 6 months previously	Persistent mildly active disease	Normal	6,000	31,000	500	Severe pancarditis, beginning 24 hours after operation, lasting 19 days, almost fatal.
	G.M.	None recognized	Apparently quiescent	Normal	10,450	20,000	167	Mild rheumatic attack with polyarthrititis beginning 48 hours postoperative.
	H.D.	Pharyngitis 10 weeks previously	Mild activity subsiding	Normal	5,900	26,250	111	Joint pains 6 days postoperative. Followed by gross hematuria.
	V.B.	Pharyngitis 6 months previously	Apparently quiescent	Normal	7,250	18,450	143	Severe epistaxis and fever 4 days post-operative.
	D.A.	Pharyngitis 8 months previously	Apparently quiescent	Normal	13,350	16,050	167	Epistaxis 2nd and 4th days postoperative.

\* These titers were the same as the preoperative titers.

rheumatism during the spring months were operated on in the late summer or fall. At that time the clinical findings and blood studies indicated that the disease process was either quiescent or subsiding. The antistreptolysin titers were all above 100 units. The findings are presented in Table II, Group C.

These five individuals experienced a rheumatic recrudescence after splenectomy. The onset of

manifestations was accompanied by leukocytosis and included pancarditis, polyarthrititis, epistaxis, hematuria and pyrexia, symptomatically relieved by salicylates. In some, the recrudescences occurred shortly after splenectomy; in others, there was a brief symptom-free period. One illustrative record is presented in Table III and Figure 1. None of these recrudescences were accompanied by a rise in antistreptolysin titer.

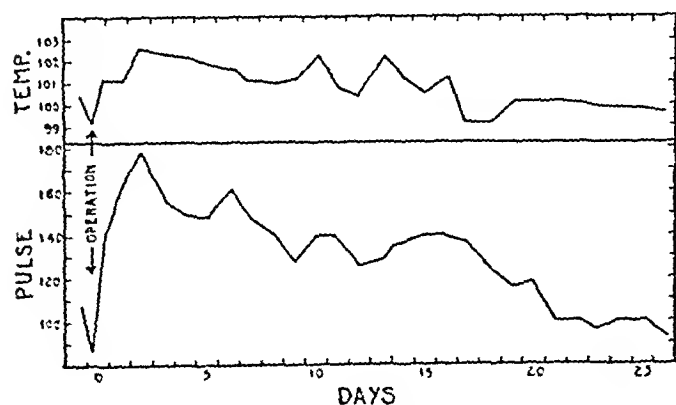


FIG. 1. DAILY MAXIMUM TEMPERATURE AND PULSE OF PATIENT D. K., DURING RHEUMATIC RECRUDESCENCE FOLLOWING SPLENECTOMY.

#### CASE HISTORIES

*D. K., Number 234004.* The patient, a girl of seven, was known to have been rheumatic since the age of four. In February, 1932, she contracted pharyngitis which was followed by a prolonged rheumatic attack. The physical examination in October showed a well nourished girl in apparently good health. There was slight enlargement of the left side of the heart and a systolic murmur at the apex transmitted to the axilla. The leukocyte count was 6,050 with 55 per cent polymorphonuclears. The blood sedimentation rate, which was not reported until after operation, was 85 mm. in one hour. Splenectomy was performed under ether anesthesia on October 11th. The operation took thirty minutes. There were a few adhesions around the upper pole of the spleen. No diffi-

TABLE III

*Clinical and laboratory findings in Patient D. K. during rheumatic recrudescence following splenectomy*

Date	Blood				Throat flora	Clinical observations	Remarks
	W.B.C.	Sedimentation rate	Anti-streptolysin titer	Precipitation reaction*			
1932							
October 11	6,000	85	500	Negative	Normal	Subsiding rheumatism—symptom free Condition apparently good Pulse 180. Heart tremendously overactive Temperature 102°, pulse 180 Apprehensive, dyspneic, orthopneic Marked pallor and intense cyanosis, cardiac pain Hyperesthesia over precordium Symptoms of cardiac insufficiency persisting with temperature 100° to 102°, pulse 110 to 160, marked anorexia and restlessness	Splenectomy October 11, 1933 Ekg. normal October 11, 1933 Operation followed within 24 hours by a fulminating, almost fatal attack of carditis. Precordial pain partially relieved by morphine and ice bag
October 12							
October 13	31,800						
October 14	31,800						
October 15							
October 17	16,100						
October 18	16,100						
October 19	22,750	120					
October 20	17,750						
October 21	20,950						
October 24	20,900				Normal	Beginning improvement characterized by less pallor, stronger pulse 100–140, temperature 99° to 101° Still listless and tired with slightest exertion, apprehensive Sudden subsidence of all cardiac symptoms Marked improvement in appetite Is much brighter, eating well, regaining strength Symptom free. Color good Temperature 99°, pulse 90  Improving rapidly  In chair, without symptoms Good appetite, excellent color Steady, rapid improvement	Ekg.—T waves inverted in all leads since October 11, 1932 Aspirin 3 grams o.d.  Ekg.—T waves inverted in all leads. Rate 130  Ekg.—T waves inverted in all leads. Rate 95  Ekg.—T <sub>1</sub> and T <sub>2</sub> upright but definitely abnormal in contour. Rate 90
October 25	40,450	110	333	Negative			
October 26	17,400						
October 27	21,900						
October 28	22,150						
October 29	13,550						
October 31	17,700	33					
November 1	15,500		333	Negative			
November 2	9,900						
November 3	13,300						
November 4	12,150						
November 5					Normal	Symptom free. Color good Temperature 99°, pulse 90  Improving rapidly  In chair, without symptoms Good appetite, excellent color Steady, rapid improvement	Ekg.—T <sub>2</sub> upright but of low amplitude
November 6							
November 7	13,250						
November 8	20,450						
November 9							
November 10	15,300	38					
November 11	14,450						
November 12	14,450						
November 13	15,850						
November 14							
November 15	15,900				Normal	Fair condition—symptom free	April 21. Discharged to Pelham Home
November 16	17,100	14					
November 17	15,500						
November 18							
November 19							
November 20							

\* Antigen used was hemolytic streptococcus nucleoprotein—fraction C19K.

culties were encountered, and the patient returned to the ward in good condition.

The first twelve hours after operation were not alarming. The pulse rate rose to 140 and the temperature to 101. The next day the picture was that of fulminating pancarditis. The patient complained of precordial pain, the pulse rose to 180; cyanosis became intense. For seven days her condition was critical. The temperature rose; drowsiness, dyspnea and orthopnea were extreme; the heart sounds became weak, pulse was almost imperceptible; cardiac signs suggested acute pericarditis; skin hyperesthesia developed over the precordial region. On October 18th she was digitalized, and on October 21st administration of aspirin was begun. On October 26th her condition changed abruptly. After being extremely ill with symptoms and signs of marked cardiac embarrassment she suddenly became comfortable. Cyanosis, pallor, nausea, cardiac pain, and drowsiness disappeared during a period of twelve hours. The intensity of the attack abated rapidly. The heart, which had been slightly enlarged and then increased tremendously in size follow-

ing splenectomy, remained grossly enlarged in the lateral diameters at all levels with greatest enlargement in the left auricular zone. The electrocardiogram, interpreted as normal on admission, showed inversion of T waves in all three leads on October 20th and October 25th. On November 9th, T<sub>1</sub> and T<sub>2</sub> had become upright but were of low amplitude and abnormal contour. She was transferred to The Pelham Home on November 21st where, because of her low myocardial reserve, it was found necessary to maintain bed rest for twelve months.

The clinical course of this patient's illness may be visualized in the accompanying temperature record, Figure 1. The laboratory findings are presented in Table III. From this record it is seen that although symptom-free, she had a high blood sedimentation rate and a high antistreptolysin titer at the time of operation. The rheumatic process was probably active although the



patient was symptom-free when the spleen was removed. The immediate development of fulminating pancarditis was accompanied by a marked leukocytosis, rise in blood sedimentation rate, increased urinary excretion of erythrocytes and a slight drop in antistreptolysin titer. The attack lasted seventeen days and subsided abruptly, accompanied by a fall in blood sedimentation rate from 110 mm. to 33 mm. The electrocardiographic changes were in accord with the clinical findings of severe carditis.

### *Examination of spleens*

Gross and microscopic examination and bacteriological cultures were made of the spleens immediately after removal. The cultures all remained sterile. The gross appearance of the spleens was essentially normal, and no differences were detected between the spleens of individuals who developed recrudescences and those who remained quiescent.

### SUMMARY

Splenectomy did not permanently modify either the immune response or the character of the rheumatic recrudescence.

Nine out of twenty apparently quiescent rheumatic subjects developed recrudescences as a direct sequel to splenectomy.

All of these nine individuals had elevated antistreptolysin titers at the time of operation, and none of them showed any increase in titer during or after the recrudescence.

This shows that following operative manipulation of antibody-producing tissue during subsiding rheumatism, an exacerbation of symptoms may develop in the absence of further rise in antistreptolysin titer.

### DISCUSSION

The authors have pointed out that the first phase of acute rheumatism is infection of the upper respiratory tract with hemolytic streptococcus. This organism usually disappears from the throat flora within a few days. Its subsequent history is unknown. There is evidence, however, that its disappearance from the pharyngeal mucosa does not mean that it has been eliminated from the body. Pure cultures of hemolytic streptococ-

cus have been recovered by the authors from the tonsils of patients whose throat flora had been free of hemolytic streptococcus for a period of months. The agent recovered from the tonsils appeared identical with the organism which had been associated with the acute respiratory infection some months prior to tonsillectomy. Furthermore, autopsy findings in an individual whose tonsils had been removed have also shown the presence of an active infectious agent weeks after its disappearance from the surface of the pharynx. In this patient, Hallahan (see (2) Plate 10, Figure 4), although the throat flora had been free of hemolytic streptococcus for several weeks before death, microscopic sections of the pharyngeal tissue showed a severe inflammatory reaction with streptococci present in typical chain formation. Furthermore, smears from the tracheal lymph nodes contained many degenerate forms which appeared to be streptococci. From the bacteriological and the histological findings it seems likely that streptococcus can maintain activity in tissues surrounding the upper respiratory tract long after its disappearance from the buccal mucosa.

The second phase of acute rheumatism is a symptom-free period about which nothing is known. This is followed by the third phase, the rheumatic attack which occurs coincidentally with the appearance of antibodies in the circulation. By the time the third phase begins, hemolytic streptococcus has usually disappeared from the pharyngeal mucosa. However, the persistence of high antistreptolysin titer values for months after the onset of a rheumatic attack is evidence for the long-continued presence of the corresponding antigen—presumably the infectious agent itself. During this period when the antistreptolysin titer is markedly elevated in the rheumatic subject there is usually clinical evidence of activity of the rheumatic process. The prolonged character of rheumatic disease and the parallel persistence of high antistreptolysin titers are in accord with the concept that the infectious agent is present.

In contrast to the typical rheumatic attack which consists of three distinct phases, another type of attack has been observed. This variety of recrudescence follows almost immediately after operative procedures in the upper respiratory tract, such as tonsillectomy or extraction of teeth. It may be associated with invasion of the blood stream

since transient bacteremia following tonsillectomy has been observed (10). Prior to the splenectomies reported above, the authors had observed this type of rheumatic recrudescence only following trauma to the pharynx or buccal cavity. It had not followed other operative procedures or trauma elsewhere in the body. Appendicectomy in ten rheumatic subjects under observation produced no rheumatic symptoms although five of these individuals had active rheumatism at the time of operation. Eleven rheumatic subjects who had severe fractures remained free of rheumatic symptoms.<sup>4</sup>

It was, therefore, not expected that recrudescences would develop following splenectomy. This operative procedure in non-rheumatic subjects is not followed by rheumatism. Since cultures of the spleens and the operative field were both sterile, blood stream invasion analogous to that following tonsillectomy need not be considered as a factor. The fact that not one of these post-splenectomy attacks was accompanied by a rise in antistreptolysin titer indicates that there was no activation of the immunity mechanism. In this respect these postoperative recrudescences differ strikingly from the typical rheumatic attack. Furthermore, they followed almost immediately after removal of the spleen, without the usual symptom-free period.

The mechanism of these rheumatic recrudescences following splenectomy must therefore be somewhat different from the normal or post-tonsillectomy types. It has been pointed out above that these nine recrudescences all occurred in individuals with high antistreptolysin titers. Only three patients with titers above 100 units escaped postsplenectomy rheumatic symptoms, and none of the patients with low titers developed any rheumatic symptoms.

When the rheumatic subject has been free of streptococcus infection for a long period of time, the antistreptolysin titer is low and presumably the antibody producing tissues are quiescent. In such patients splenectomy produced no untoward results. In contrast, when rheumatic subjects have had fairly recent hemolytic streptococcus in-

fections and still have high antistreptolysin titers the spleens probably contain streptococcus products and are active in the production of antibody (8). In such patients splenectomy was followed by recrudescence.

In order to account for the occurrence of these postsplenectomy attacks, the following hypothesis has been formulated: that the spleen of the rheumatic subject when actively engaged in the production of antibodies to hemolytic streptococcus contains some substance which can be released into the circulation. This substance, whether of bacterial or human origin, damages certain mesodermal tissues either directly or indirectly. In the course of an ordinary rheumatic attack it is released gradually during the period of immune response of the antibody-producing tissues, and gives rise to symptoms over a long period of time. In the attacks induced by splenectomy, the material was liberated suddenly by operative manipulation, producing almost immediate symptoms. On the other hand, in quiescent rheumatic subjects whose titers were low, and whose spleens were presumably inactive insofar as antibody production was concerned, this substance was either not present or, if its action is indirect, did not give rise to a toxic derivative. The intensity of the recrudescences following splenectomy paralleled the activity of the antibody-producing organs, as indicated by the antistreptolysin titers. Under this hypothesis, manipulation of the "active" spleens released into the circulation some substance, presumably non-viable, which was effective in initiating the clinical manifestations of rheumatic fever. The nature of this substance remains to be determined.

#### GENERAL CONCLUSIONS

The findings presented in earlier papers of this series (1, 2) led to two conclusions. First, both influenza virus and one type of hemolytic streptococcus were ineffective in initiating recrudescences in a rheumatic colony. This strain of streptococcus did not produce soluble toxin, and although present in the throat flora was not associated with acute infection. Second, another type of hemolytic streptococcus was highly effective in initiating recrudescences in 14 out of 16 rheumatic members of this same colony. This strain

<sup>4</sup> One other rheumatic patient had a Pott's fracture a few days after contracting hemolytic streptococcus pharyngitis. Two weeks later she developed acute rheumatism with high antistreptolysin titer.

was a strong toxin producer, was associated with acute infection of the upper respiratory tract and was a single serological type. In addition to infection with an effective strain of hemolytic streptococcus, two other factors contributed to the development and character of this epidemic of acute rheumatism. These factors were first, the disease pattern peculiar to each rheumatic subject, and second, the intensity of the immune response.

Next it was pointed out (3) that most strains of hemolytic streptococcus which are effective in producing acute rheumatism form soluble toxin and streptolysin and are indistinguishable from scarlatinal strains. Most of the strains associated with throat infections which are ineffective in initiating rheumatic attacks form little or no soluble toxin. This finding, in conjunction with earlier studies (11), indicates a close relationship between scarlet fever, acute rheumatism and toxin-producing strains of hemolytic streptococcus.

The findings presented in the fifth paper of the present series (4) demonstrated that neither active nor passive immunization to hemolytic streptococcus inhibited the development of the rheumatic process. The observations indicated that the development of rheumatic activity depends not only upon infection with an erythrogenic strain but also upon the host's immune response to such infection.

Next it was pointed out (5) that there is a close time relationship between the antistreptolysin rise and the onset of rheumatic activity. A small number of rheumatic subjects were observed to be infected with strains of hemolytic streptococcus which produced strong toxin and streptolysin, and nevertheless fail to develop a significant rise in antistreptolysin titer. These individuals also failed to develop a rheumatic attack. The authors interpret this fact to mean that in addition to infection with an effective strain, stimulation of the host sufficient to produce an antibody response is essential to initiation of rheumatic activity. It seemed that the character of the antibody response played a large part in determining the type of the rheumatic attack.

Finally, the observations made in the present paper indicate that in the patients who escaped postsplenectomy rheumatic recrudescences, the antibody-producing tissue was presumably quies-

cent; and that in those patients who developed acute rheumatism following splenectomy, the antibody-producing tissue was probably in a state of activity. Likewise, as was already pointed out, in the subjects who escape recrudescences following infection with hemolytic streptococcus, the antibody-producing tissue presumably remains quiescent; and in those subjects who develop acute rheumatism following hemolytic streptococcus infection, the antibody-producing tissue is activated. These two sets of observations together show that acute rheumatism may follow either activation of the antibody-producing system by hemolytic streptococcus or operative manipulation of this system during the period in which it is producing immune bodies to hemolytic streptococcus.

It is the authors' conception that rheumatic disease is the result of the following sequence of events: (1) Infection with toxin-producing strains of hemolytic streptococcus initiates a process peculiar to rheumatic subjects; (2) In the course of this process a substance is released, presumably from the antibody producing tissues, which either directly or indirectly alters mesodermal structures. This substance is probably not the infecting organism, and at the present time there is no evidence to suggest that it is viable; (3) The release of this toxic substance seems to take place only when there is an immune response to hemolytic streptococcus.

In conclusion, the collected evidence indicates that activity of the rheumatic process depends not only upon effectiveness of the infecting strain of hemolytic streptococcus but also upon the intensity of the immune response of the rheumatic subject to this bacterial agent.

To the three nurses, Lucille Miller, Mary Kellr and Ruth Colby, the care of the patients was entrusted. Dr. Edward J. Donovan performed the splenectomies. Dr. Lucille Moore and Miss Eleanor M. Kapp assisted the authors in many ways. The advice of Dr. Alphonse R. Dochez was invaluable. With the help of these associates and with the assistance of The W. K. Kellogg Foundation the authors have been able to conduct the present studies.

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# A STUDY OF GASTRIC PEPSIN IN VARIOUS DISEASES

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The study of gastric pepsin has resulted in rather conflicting reports in the literature. Its significance in estimating the secretory capacity of the stomach has apparently not been widely recognized. In studying the causes of chronicity of peptic ulcer, we have gained the impression that increased pepsin concentration in the gastric contents plays a rôle in delaying healing of artificial defects in the stomach (1). In view of these facts, it was felt desirable to study the secretion of pepsin in patients with and without disease of the stomach.

The results are reported in this paper.

## METHOD

The gastric contents were obtained one hour after an Ewald meal, in most instances without a fasting expression. In a small group fasting samples were taken before the test meal. The juice was filtered immediately, diluted and kept in the ice box. The pepsin determinations were done within three hours as a rule, though values for the diluted specimens were found to remain unchanged up to three days.

Free and total acidity were titrated with 1 cc. of the filtered juice against N/100 sodium hydroxide. Toepfer's reagent was used as the indicator for free acid and phenolphthalein solution for total acid.

The peptic activity of the gastric contents was determined by the method of Anson and Mirsky (2). This depends upon the digestion of hemoglobin by pepsin, the end-product being tyrosine. The procedure in detail is as follows. One cubic centimeter of the filtered gastric juice is diluted with N/10 HCl, the dilution being usually between 1:5 and 1:15 and selected in most instances by the acidity of the specimen. The final colorimetric reading must fall between 10 and 30 mm., the standard being at 20 mm. At times the preliminary readings do not fall within this range; then the test must be repeated with a different

dilution; but with experience this is seldom necessary.

To 5 cc. of 2 per cent carbon monoxide hemoglobin in .06 N HCl in a test tube is added 1 cc. of the diluted gastric juice. Digestion is allowed to proceed for five minutes in a constant temperature water bath at 35.5° C., after which 10 cc. of 4 per cent trichloroacetic acid are added, which stops the digestion by precipitating the remaining hemoglobin. The mixture is filtered through fine paper. To 3 cc. of the clear filtrate in a 50 cc. Erlenmeyer flask are added 20 cc. of distilled water, 1 cc. of 3.85 N sodium hydroxide and 1 cc. of phenol reagent. The standard consists of 20 cc. of distilled water, 3 cc. of N/10 HCl containing 0.15 mgm. of tyrosine, 1 cc. of 3.85 N sodium hydroxide and 1 cc. of phenol reagent. The blue colors are compared after from five to ten minutes.

In order to determine the amount of coloring matter already in the specimen, a blank is run through with the original as follows: hemoglobin 5 cc. + 10 cc. trichloroacetic acid + 1 cc. diluted juice, given five minutes digestion. To 3 cc. of the filtrate from this are added 14 cc. distilled water, 3 cc. N/10 NaOH, 3 cc. of the standard tyrosine solution, 1 cc. 3.85 NaOH and 1 cc. phenol reagent. The difference between this solution and the standard gives the amount of extraneous coloring matter in the specimen being tested. This is subtracted from the original and the result, multiplied by the dilution, gives the units of pepsin, P. U., of the original specimen. The formula is:  $P. U. = (0.0195/x) - 0.000147$  where  $x$  is the colorimeter reading. In practice, a table containing all the values for readings between 10 and 30 is used.

In order to convert these results into more significant values, we have translated them into milligrams of pepsin (1:4,000) per cubic centimeter as suggested by Helmer, Fouts, and Zervas (3). Repeated determinations on a 0.1 per cent solution of commercial pepsin (Armour & Co.,

1:4,000) have given an average P. U. of 0.001700.

### RESULTS

For purposes of tabulation the cases have been divided into six groups as follows:

*Miscellaneous group* (Table I). This includes 28 cases on the general medical and surgical wards

TABLE I

*Secretion of pepsin in response to an Ewald meal in 27 patients with miscellaneous conditions*

Case number	Age	Free acidity	Total acidity	Pepsin	Diagnosis
	years			mgm. per cc. of gastric juice	
1	29	25	57	2.1	Not sick
2	24	28	50	2.8	Rheumatic heart disease
3	19	56	79	4.4	Convalescent trichiniasis
4	17	26	47	4.0	Convalescent pleurisy with effusion
5	35	45	80	5.5	Pulmonary tuberculosis
6		65	85	8.9	Neurosis
7	49	43	72	2.6	Carcinoma of pancreas
8	22	15	43	5.2	Pulmonary tuberculosis
9	72	0	20	5.8	Anomaly of duodenum
10	45	6	27	2.8	Arteriosclerotic heart disease
11	57	35	56	4.6	Non-tropical sprue
12	32	41	65	9.8	Chronic osteomyelitis
13	33	62	91	3.2	Chronic erysipelas of leg
14	18	50	74	7.1	Gastric neurosis
15	63	28	41	3.8	Arteriosclerosis
16	30	26	49	1.9	Dermatitis herpetiformis
17	53	62	86	6.9	Fistula in ano
18	24	31	74	4.5	Hand infection
19	27	21	43	3.4	Post-appendectomy—convalescent
20	41	17	41	3.2	Secondary anemia, cause unknown
21	49	19	52	2.0	Constipation
22	28	4	30	2.0	Pneumonia. Convalescent for 2 weeks
23	39	60	80	5.8	Perianal abscess
24	54	33	51	0.8	Aplastic anemia
25	59	0		0.7	Undiagnosed deficiency syndrome
26	40	0		0.4	Secondary anemia
27	43	0	7	0.1	Hypochromic anemia

with no known disease of the stomach or duodenum. The median value for these cases is 3.6 mgm. pepsin per cc. Interesting values are seen in Case 9 with achlorhydria and slightly high pepsin; Case 11 with sprue-like deficiency, hyperchromic anemia, normal acid and slightly high pepsin; Case 24 with an aplastic type of anemia, normal acid and very low pepsin; and Cases 25, 26, and 27 with anemia, achlorhydria and low pepsin concentrations. The last four cases fall within the range seen in pernicious anemia (see Table V). The mean free acid value for this miscellaneous group was 31.

*Duodenal ulcer group* (Table II). These were patients who had not been operated upon but were

TABLE II

*Secretion of pepsin in response to an Ewald meal in thirty-three patients with duodenal ulcer*

Case number	Age	Free acidity	Total acidity	Pepsin
	years			mgm. per cc. of gastric juice
1...	42	60	72	4.0
2...	64	78	88	4.7
3...	31	70	84	5.1
4...	31	50	86	4.0
5...	44	67	96	6.0
6...	59	77	97	11.0
7...	36	71	93	4.7
8...	37	69	84	9.9
9...	26	50	72	6.6
10...	46	34	51	8.7
11...	43	90	111	8.7
12...	49	16	29	2.1
13...	37	15	41	3.9
14...	42	57	73	7.6
15...	25	26	58	6.9
16...	35	52	89	8.1
17...	24	45	74	6.9
18...	42	41	68	7.8
19...	36	64	96	7.2
20...	29	62	93	7.1
21...	34	37	64	7.6
22...	29	15	40	6.1
23...	52	10	32	4.7
24...	32	64	83	6.8
25...	62	41	78	10.7
26...	18	36	61	5.1
27...	50	44	75	7.9
28...	49	22	42	3.0
29...	33	37	67	5.3
30...	29	33	52	5.5
31...	35	77	95	8.4
32...	49	47	71	11.7
33...	36	43	82	9.1

undergoing medical treatment in the hospital. All except one (Case 11) were having symptoms at the time of observation. The median value for this group of 33 cases was 6.75 mgm. of pepsin per cc. or slightly over twice that of the miscellaneous group. Variations were fairly marked, ranging from 2.1 to 11.7 mgm. The mean free acid value was 47.5.

*Postoperative ulcer cases* (Table III). There were only five patients in this group, with values between 0 and 3.4 mgm. Three had been subjected to partial gastrectomy 19 years, 15 months and 3 weeks before, respectively. The latter case showed no pepsin. Cases 1 and 2 had had previous short-circuiting operations, and these, as well as Case 4, were suffering from ulcer-like symptoms at the time the analyses were done.

*Carcinoma of the stomach* (Table IV). This group comprises six patients. The diagnosis was

TABLE III

*Secretion of pepsin in response to an Ewald meal in five postoperative ulcer patients*

Case number	Age	Free acidity	Total acidity	Pepsin	Diagnosis
	years			mgm. per cc. gastric juice	
1...	39	18	32	3.4	Old gastro-enterostomy (10 years). Gastric ulcer. Jejunal ulcer (?) Old duodenal ulcer.
2...	29	14	42	2.5	Gastro-enterostomy and exclusion of antrum (9 months). Jejunal ulcer
3...	37	0	7	0	Sub-total gastrectomy for duodenal ulcer (3 weeks).
4...	41	0	3	0.5	Partial gastrectomy for duodenal ulcer (15 months). Jejunal ulcer (?).
5...	29	12	37	1.2	Partial gastrectomy for gastric ulcer (19 years).

TABLE IV

*Pepsin values in six cases of carcinoma of stomach after an Ewald meal*

Case number	Age	Free acidity	Total acidity	Pepsin
	years			mgm. per cc. of gastric juice
1....	53	0	6	1.0
2....	59	0	49	0.1
3....	65	0	8	0.4
4....	67	0	13	0.5
5....	59	5	21	6.5
6....	71	0	17	1.0

subsequently confirmed by operation in all except Case 1 in which the x-ray and clinical picture were typical (the patient refused operation). Case 5 was the only one showing free acid, accompanied by an elevated pepsin value. In the others the pepsin was quite low.

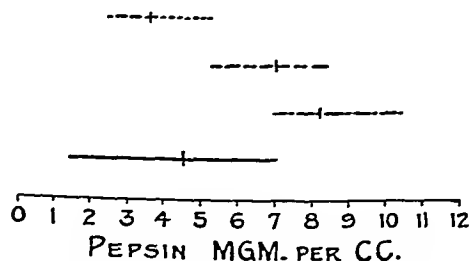


FIG. 1. COMPARISON OF MEDIAN AND INTERQUARTILE RANGES OF AMOUNT OF PEPSIN SECRETION IN MG. PER CC. OF GASTRIC JUICE IN PATIENTS WITH MISCELLANEOUS CONDITIONS (.....), IN PATIENTS WITH DUODENAL ULCER (-----), AND IN THE TOTAL GROUP (———), IN RESPONSE TO AN EWALD MEAL; AND IN FASTING PATIENTS (— · — · —) (THE TOTAL GROUP DOES NOT INCLUDE THE FASTING PATIENTS).

*Pernicious anemia* (Table V). This includes ten patients, all with achlorhydria and very low total acid. The pepsin values range from 0 to 0.5 mgm., nine cases showing only traces.

TABLE V

*Pepsin secretion in ten cases of pernicious anemia after an Ewald meal*

Case number	Age	Free acidity	Total acidity	Pepsin
	years			mgm. per cc. of gastric juice
1...	51	0	2	0
2...	35	0	5	0.1
3...	59	0	2	0.1
4...	35	0	4	0.1
5...	57	0	4	0.2
6...	42	0	3	0.1
7...	32	0	3	0.3
8...	65	0	4	0.1
9...	55	0	1	0.5
10...	48	0	3	0

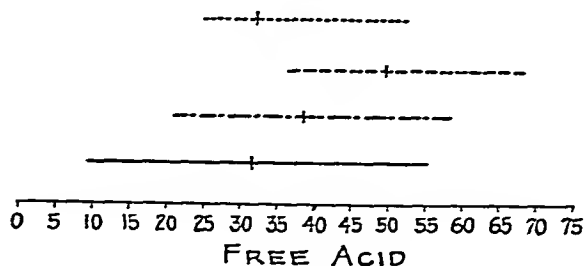


FIG. 2. COMPARISON OF MEDIAN AND INTERQUARTILE RANGES OF AMOUNT OF FREE ACID (CC. N/10 PER 100 CC.) IN PATIENTS WITH MISCELLANEOUS CONDITIONS (.....), IN PATIENTS WITH DUODENAL ULCERS (-----), AND IN THE TOTAL GROUP (———), IN RESPONSE TO AN EWALD MEAL; AND IN FASTING PATIENTS (— · — · —) (THE TOTAL GROUP DOES NOT INCLUDE FASTING PATIENTS).



*Fasting expressions* (Table VI). These 16 observations are presented to show the extreme variation in fasting pepsin concentration. Ex-

TABLE VI

*Pepsin content of fasting gastric secretion in sixteen individuals*

Case number	Age	Free acidity	Total acidity	Pepsin	Diagnosis
	<i>years</i>			<i>mgm. per cc. of gastric juice</i>	
1	44	128	142	12.1	Duodenal ulcer
2	31	26	36	8.0	Duodenal ulcer
3	31	43	51	7.4	Duodenal ulcer
4	36	18	26	5.8	Duodenal ulcer
5	37	59	71	9.8	Duodenal ulcer
6	55	55	70	6.1	Carcinoma of stomach
7	29	58	88	8.1	Not sick
8	19	11	27	4.6	Convalescent trichiniasis
9	56	0	4	0.5	Hernia
10	40	48	63	10.7	Hernia
11	38	30	50	6.4	Inguinal hernia
12	16	0	6	1.0	Hernia
13	35	32	50	7.9	Duodenal ulcer
14	60	60	84	9.3	Duodenal ulcer
15	35	24	40	21.9	Pulmonary tuberculosis
16	38	57	78	11.8	Neurosis

cluding the two cases with achlorhydria and low pepsin, the figures vary from 4.6 to 21.9 mgm., the median value for the group being 7.75 mgm. per cc. Obviously, a fasting expression is subject to so many inconstant factors that a determination of its content seems to be of relatively little value.

For the 81 cases examined one hour after an Ewald meal the median value for pepsin was 3.9 mgm. per cc. and for free acid 33.

#### DISCUSSION

In considering the secretion of pepsin by the stomach, it seems important to point out the practical differences between peptic activity and the concentration of pepsin. It is known that the optimum pH for peptic activity is between 1.9 and 2.3, and that outside of this range digestive action rapidly diminishes. It will be noted that in the method we have used, the pH of the digestion mixture may vary slightly with differences in acidity of the juices to be tested. But since the hemoglobin used is in solution in approximately

.06 N HCl, and 5 cc. are used for 1 cc. of gastric juice, the change in pH is not outside the optimum range for peptic activity. Substantiating this, we have found that a commercial preparation of pepsin gives the same values whether dissolved in N/10 HCl, N/100 HCl or in distilled water.

Our results measure the amount of pepsin secreted rather than the peptic activity in vivo, since artificial conditions are created whereby the pH of the original juice as ordinarily encountered makes little or no difference in the result. Thus in cases of pernicious anemia, where the acidity is very low, the juice might show no peptic activity as found in the stomach but when analyzed as above shows appreciable amounts of pepsin. These facts may also explain why the range of values in our cases do not vary as much as those of other workers (4). A fairly accurate idea of the activity of any given juice in vivo can be obtained after considering the acidity and the amount of pepsin present.

Our results show a fairly high coefficient of correlation between the acid and pepsin secretion, viz. 0.74 for the entire group. In general, a high acid is likely to be accompanied by high pepsin and vice versa, but frequent exceptions are found. These findings are in general similar to those of Helmer, Fouts, and Zervas (3), though these investigators lay more stress upon the frequent instances among their cases of dissociation of the acid and enzyme content.

The very low values for pepsin in pernicious anemia also confirm the results obtained by these investigators (5), though they used histamine as a stimulus.

As to the choice of stimulus used in studying pepsin secretion, it is known from the early work of Pavlov that among the foods bread is one of the most powerful stimulants of pepsin secretion. There is still some controversy as to whether histamine stimulates secretion of pepsin or not. Vineberg and Babkin (6), as well as Gilman and Cowgill (7), feel that it does not, but Pollard and Bloomfield (8) have found an increase in the total pepsin output following the administration of histamine.

From this series of cases it seems clear that, in general, patients with duodenal ulcer secrete more pepsin than do those without ulcer. This

is in accord with the work of Vanzant, Osterberg et al. (4), though we have not found as marked variations from the normal as they reported. We have not followed our patients long enough as yet to determine whether a high value for pepsin makes the prognosis less favorable, as these investigators believe. On the other hand, Polland and Bloomfield (9), in a small group of cases, noted no significant differences between the value for pepsin in cases with duodenal ulcer and in controls.

It would seem that pepsin determination in cases of carcinoma of the stomach is of little help in diagnosis, since it apparently reflects only the usual picture of hyposecretion as seen in the acid values.

#### SUMMARY AND CONCLUSIONS

1. A series of pepsin determinations, done according to the hemoglobin digestion method of Anson and Mirsky (2), is reported.

2. With the Ewald test meal the following results were obtained:

(a) In a miscellaneous group of patients the median value for pepsin was 3.6 mgm. per cc. of gastric juice and the median value for free acid was 31.

(b) In a group of duodenal ulcers the median value for pepsin was 6.75 mgm. and for free acid 47.5.

(c) In a small series of five postoperative ulcers the pepsin ranged from 0 to 3.4 mgm.

(d) In six cases with carcinoma of the stomach all, except one, had low concentrations of pepsin.

(e) Patients with pernicious anemia showed only traces of pepsin.

(f) The median value for pepsin for the entire group was 3.9 mgm. per cc. and for free acid was 33.

3. Fasting gastric expressions gave rather marked variations in pepsin concentration, the median value being 7.75 mgm. per cc.

4. A high correlation existed between the acid and pepsin secretion in response to the test meal, though exceptions were not infrequent.

We are indebted to Dr. Michael Heidelberger and staff for assistance in preparing the chemical reagents used in the pepsin determinations.

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# THE EFFECT OF SPLANCHNIC NERVE RESECTION AND SYMPATHETIC GANGLIONECTOMY IN A CASE OF PAROXYSMAL HEMOGLOBINURIA

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Several observers have directed attention to the frequent occurrence of vasomotor disturbances in patients with paroxysmal hemoglobinuria, and a few (1, 2, 3, 4, 5) have suggested that these disturbances are of importance in the pathogenesis of the disease. The purpose of this communication is to present observations on a patient with paroxysmal hemoglobinuria which demonstrate that the sympathetic nervous system played a significant, although not the primary, rôle in the production of the attacks.

## REPORT OF A CASE

*History.* The patient, a white man 38 years of age, a painter, was first seen on December 4, 1933. The past medical and family histories were irrelevant. There was no history of syphilitic infection or of antiluetic treatment. The patient had lived in Ohio (temperate climate) for 26 years, and his occupation had not entailed more than moderate exposure to cold weather.

For three years before coming to the Clinic, the patient had experienced frequent attacks of burning pain in the epigastrium and left upper abdominal quadrant. In November, 1932, he first noticed that exposure to cold weather caused numbness and burning pain in the fingers and toes and that the fingers at the same time became cyanotic. The ears and nose also became cyanotic but were not painful. It was observed also that exposure to cold sufficient to cause these changes was followed invariably by the passage of dark red, reddish-brown or almost black urine during the succeeding two to four hours. Hemoglobinuria had never been preceded or accompanied by a chill and the patient had never felt feverish.

During the warmer months of 1933, no attacks of hemoglobinuria were experienced, but with the onset of cold weather, paroxysms again occurred and were always preceded by the changes in the fingers, toes, ears and nose described above. Persistent weakness had been a complaint since the first appearance of hemoglobinuria and had prevented the patient from working.

*Examination.* The patient was well developed but somewhat pale. The pupils were regular and reacted normally. There was no gingival lead line. The heart and lungs were normal on percussion and auscultation. The liver, spleen and kidneys were not palpable, and there was no abdominal tenderness. Neurologic exami-

nation gave entirely normal findings. The blood pressure was 140 mm. Hg systolic and 80 mm. Hg diastolic.

Specimens of urine collected at times when the patient had not been exposed to cold contained no albumin or sugar and showed nothing abnormal on microscopic examination.

Examination of the blood revealed extreme agglutination of the erythrocytes whenever the specimens were allowed to cool below body temperature. Clumping occurred so rapidly that cover glass smears made as quickly as possible contained grossly visible aggregations of cells. This auto-agglutination made it impossible to obtain red blood cell counts by the ordinary method, and in order to obtain satisfactory counts, it was necessary to use warm diluting fluid, warm pipettes and a warm counting chamber. The erythrocyte count made with these precautions was 3,800,000 per cu. mm. The hemoglobin content was 10.5 grams per 100 cc., and differential counts of stained smears gave a normal distribution of cell types. The patient's blood belonged to Group III (Jansky). Fragility tests showed hemolysis to begin in 0.40 per cent sodium chloride solution and to be complete in 0.34 per cent solution.

The Wassermann reaction of the blood was negative with plain alcoholic, cholesterinized and acetone insoluble antigens. The Kahn reaction of the blood also was negative. The Wassermann and Kahn reactions of the spinal fluid and the colloidal gold test were negative.

## Special studies

*Donath and Landsteiner reaction.* One of the characteristic features of paroxysmal hemoglobinuria is the presence in the patient's blood serum of an hemolysin which unites with the red blood cells of the patient or of other persons only at low temperatures. Hemolysis occurs when the mixture subsequently is warmed, providing complement is present. This is the Donath and Landsteiner reaction (6). The results of this test in our patient are presented in Table I. The test was performed on four occasions before the patient was subjected to operation, and similar results were obtained each time.

*Induction of attacks of hemoglobinuria.* Hemoglobinuria could be produced at will by the application of an ice pack extending from the feet to the level of the anterior superior spine of the ilium or to the ensiform cartilage for from 20 to 40 minutes (Tables II and IV). Specimens of urine collected 30 minutes, one hour and two hours after removal of the pack varied in color from light red to deep reddish brown and contained a large

TABLE V

*Observations after resection of left splanchnic nerves and removal of left first lumbar ganglion (January 22, 1934)*

Date	Procedure	Hemo-glo-binuria	Hemo-glo-binemia*
<i>1934</i>			
February 1	Ice pack, feet to anterior superior spine of ilium, 40 minutes	0	0
February 5	Ice pack, feet to anterior superior spine of ilium, 40 minutes	0	0
February 6	Exposure in room at 49° F., 1 hour	0	0
February 7	Ice pack, feet to ensiform cartilage, 40 minutes	0	0
February 10	Ice pack, feet to ensiform cartilage, 40 minutes	0	0
February 12	Ice pack, feet to ensiform cartilage, 40 minutes	0	0
February 21	Ice pack, feet to ensiform cartilage, 40 minutes	0	0
March 20	Ice pack, feet to ensiform cartilage, 40 minutes	+	+
March 21			
March (a.m.)			
March 22	Ice pack to left leg, 40 minutes	0	0
March (p.m.)			
March 21			
March (p.m.)			
March 22	Ice pack to right leg, 40 minutes	+	+
March (a.m.)			
March 23	Exposure in room at 45° F., 1 hour	+	+
March 24	Spinal anesthesia followed by exposure in room at 40° F., 1 hour	0	0
March 25	Ice pack, feet to ensiform cartilage, 40 minutes	+	+
March 27	Exposure in room at 42° F., 1 hour	+	+

\* Blood collected one hour after end of exposure.

and the foot cyanotic. Slight cyanosis of the ears and nose also developed. Neither hemoglobinuria nor hemoglobinemia occurred (Table V). On the sixteenth, nineteenth and twenty-first days after operation, an ice pack was applied from the feet to the level of the ensiform cartilage for 40 minutes. On no occasion did hemoglobinuria or hemoglobinemia occur (Table V).

On the fifteenth day after operation, the Donath and Landsteiner test was repeated, and for the first time a negative reaction was obtained. The test was repeated three days later with the same result. At this time, tests also were made in which the mixtures of cells, serum and complement were chilled at 0° C. for 5, 30 and 60 minutes instead of for the customary 10 minutes, but in none of these tests did hemolysis occur. It also was demonstrated that the absence of hemolysis was not due to the development of anti-complementary properties (2) in the patient's serum.

The patient was discharged from the hospital on the twenty-third day after operation. It required about ninety minutes for him to reach his home by automobile, and the temperature during this time was approximately 49° F. The nose and ears were cyanotic when he arrived home, but hemoglobinuria did not occur. Two days later, he returned for observation. The outside temperature at this time was 18° F., and it took about ninety minutes to reach the Clinic. On arrival, the ears and nose were cyanotic, but specimens of urine passed immediately and 30 minutes later were perfectly clear. The patient complained of weakness, anorexia and fre-

quent pain in the epigastrium and upper left quadrant of the abdomen. After leaving the Clinic, he rode for over two hours in an automobile, and the right foot and both hands became extremely cold and painful. Two hours after arriving home, the patient passed a specimen of reddish urine. Five days later (February 21), an ice pack was applied from the feet to the level of the ensiform cartilage for 40 minutes and failed to cause hemoglobinuria or hemoglobinemia (Table V).

On March 2 and March 19 exposure for one hour out-of-doors, fully dressed, at 44° F. and 40° F. respectively caused spontaneous attacks of hemoglobinuria. On March 20, an ice pack from the feet to the level of the ensiform cartilage for 40 minutes caused hemoglobinuria and hemoglobinemia. The experiment was repeated five days later with the same result (Table V).

On the morning of March 21, an ice pack was applied to the left leg (side of operation) from the foot to the groin for 40 minutes and did not cause hemoglobinuria or hemoglobinemia. In the afternoon, a similar pack was applied to the right leg. Specimens of urine collected one-half hour and one hour after removal of the ice had a light reddish tint and gave a positive benzidine reaction. Faint hemolysis was present in a blood specimen collected one hour after removing the ice. On the following day the same procedures were carried out with the variation that the pack was applied to the right leg in the morning and to the left in the afternoon. Hemoglobinuria and hemoglobinemia again occurred after applying ice to the right leg and again failed to occur after exposure of the left leg (Table V).

On March 23 and 27, the patient was exposed for one hour without clothing but with a sheet covering the trunk in a room at 45° F. and 42° F. respectively. Hemoglobinuria and hemoglobinemia occurred on both occasions after removal from the room. On March 24, spinal anesthesia was induced by the administration of 150 mgm. of spinocain. When anesthesia had extended mid-way between the costal margin and the nipple line, the patient was transferred to a room at 40° F. and exposed as before for one hour. Neither hemoglobinuria nor hemoglobinemia occurred (Table V).

*Resection of the right splanchnic nerves and first and second right lumbar sympathetic ganglia.* In view of these observations, resection of the right splanchnic nerves was performed on March 29 together with removal of the first and second right lumbar ganglia. On the thirteenth and twenty-third days after the operation, ice packs were applied from the feet to the level of the ensiform cartilage for 40 minutes, and hemoglobinuria did not occur. Subsequent experiments of this kind were made at approximately monthly intervals, and it was not until September 20, 1934, nearly six months after the second operation, that hemoglobinuria followed the application of an ice pack.

*Cervicodorsal and lumbar sympathetic ganglionectomy.* During the summer of 1934, the patient continued to complain of persistent weakness and of frequent pain in

the upper abdomen. In addition, on cool mornings, cyanosis of the nose, ears and fingers developed together with intense burning pain in the fingers. On September 24, bilateral cervicodorsal ganglionectomy was performed, and on October 19, complete bilateral lumbar ganglionectomy was carried out. These procedures did not prevent the production of hemoglobinuria by ice packs, although for a few months after the last operation, a twenty-minute pack to the level of the anterior superior spine of the ilium failed to cause an attack; whereas immediately before the operation, similar packs had caused slight hemoglobinuria.

The patient experienced but three mild spontaneous attacks of hemoglobinuria during the winter of 1934-1935 in spite of frequent exposure to cold weather. At present, exposure to cold still causes cyanosis of the nose and ears but does not cause pain in the fingers or toes. Ice packs invariably are followed by hemoglobinuria but the attacks are somewhat less severe than before the first operation. Although the patient complains much less than formerly of weakness, he still is unable to do more than light work. The Donath and Landsteiner reaction has been repeated on seventeen occasions since the first operation and has been negative each time. Numerous tests also have been made in which 10, 25 and 50 per cent cell suspensions were employed instead of the usual 5 per cent suspension, and neither this procedure nor the use of sensitization periods ranging from 5 to 60 minutes have given a single positive reaction. The titer of the auto-agglutinins in the blood serum has remained unchanged (1:1280) throughout the period of observation. On May 3, 1935 spinal anesthesia was induced to the level of the nipples and an ice pack was applied from the feet to the level of the ensiform cartilage for 40 minutes. Hemoglobinuria was produced.

#### COMMENT

The patient studied in the present investigation showed two important variations from the usual case of paroxysmal hemoglobinuria. In the first place, there was no clinical or laboratory evidence of syphilis. Donath and Landsteiner (1) reported that there was evidence of luetic infection in 95 of 99 cases of paroxysmal hemoglobinuria recorded in the literature between 1906 and 1925. In the second place, the blood serum contained auto-agglutinins in high titer. A similar phenomenon has been reported in other cases of paroxysmal hemoglobinuria (9, 10, 11) but the association is decidedly unusual. The extent to which these two variations influenced the results of the study cannot be stated.

The fact that spinal anesthesia originally prevented the production of hemoglobinuria by ice packs suggested at first that alterations in cu-

taneous blood flow induced by the anesthesia prevented adequate cooling of the blood. The coldness of the parts exposed to the ice argued against this interpretation, however, and the disappearance of the characteristic hemolysin after the first operation demonstrated that the relationship between the sympathetic system and the disease was of a more fundamental nature.

In patients with paroxysmal hemoglobinuria due to syphilis, antiluetic treatment usually causes a gradual diminution in the titer of the hemolysin and cessation of spontaneous attacks of hemoglobinuria in a few months. In only one of ten patients treated by Kumagai and Namba (4), however, did the hemolysin disappear entirely, and then not until some time between the third and fifth years after treatment was begun. Similarly, although antiluetic therapy resulted in cessation of attacks in three of Mackenzie's five patients (8), the Donath and Landsteiner reaction became negative in but one of these and then only after approximately four years' treatment. In contrast to these results was the promptness with which the Donath and Landsteiner reaction became negative in our patient after the first operation.

We are convinced that the negative reactions obtained were not due to technical errors. Fluctuation in the complement of the patient's serum could not have been responsible since fresh complement was supplied in all tests throughout the investigation. It was demonstrated repeatedly also that the negative results were not due to the development of anticomplementary properties in the serum. Furthermore, on numerous occasions the tests were made with erythrocyte suspensions of various dilutions and with sensitization periods ranging from five minutes to one hour without in any way influencing the results. It therefore seems certain that resection of the left splanchnic nerves and left first lumbar ganglion caused an actual disappearance of the hemolysin from the blood serum.

After the relapse following the first operation, hemoglobinuria could be induced by applying an ice pack to the right leg (the unoperated side) but not by applying ice to the left leg. The results of these experiments at first appeared contradictory to the earlier failure to prevent arti-

ficial attacks of hemoglobinuria by novocain injection of the lumbar sympathetic chains. It is to be remembered, however, that the latter observations were made while the blood serum contained the specific hemolysin, while the former were made after the hemolysin had disappeared.

The recurrence of spontaneous attacks of hemoglobinuria and of the positive response to ice packs after the first operation is difficult to explain in view of the fact that this occurred in the absence of demonstrable circulating hemolysin. It is probable that in patients with paroxysmal hemoglobinuria due to syphilis, attacks do not occur in the absence of hemolysin (4). It occurred to us that exposure of the body to cold might result in local production of hemolysin in tissues having an intact sympathetic nerve supply. Hemolysin could not be demonstrated by the usual technique, however, in specimens of blood collected one hour after removal of an ice pack, even though hemoglobinuria and hemoglobinemia were present at the time. Another possibility is that the persistent auto-agglutinins in the patient's serum are concerned in some way with the production of the attacks of hemoglobinuria.

The results of the investigation indicate that the sympathetic nervous system originally played a significant contributory rôle in the production of the attacks of hemoglobinuria. That this rôle was not of primary importance was demonstrated by the recurrence of hemoglobinuria after the left and right splanchnic nerves had been resected and by the fact that, in contrast to its earlier effect, spinal anesthesia no longer prevents the induction of hemoglobinuria by ice packs. The conclusions seem warranted, therefore, that, in the patient studied, paroxysmal hemoglobinuria resulted from a pathologic state in the tissue cells themselves, and that this pathologic state either produced or was accompanied by disturbances in sympathetic innervation which accentuated the condition. Resection of the splanchnic nerves and upper lumbar sympathetic ganglia relieved the sympathetic disturbances and temporarily prevented the occurrence of hemoglobinuria. The recurrence of paroxysms of hemoglobinuria probably was due to progression of the underlying pathologic process, and although the patient's present condition is improved as compared to his

original state, it is doubtful whether this improvement will be permanent.

#### SUMMARY AND CONCLUSIONS

1. In a patient with paroxysmal hemoglobinuria who presented no clinical or laboratory evidence of syphilis, hemoglobinuria could be produced at will by the application of ice packs from the feet to the level of the anterior superior spine of the ilium or ensiform cartilage.

2. During spinal anesthesia, ice packs did not cause hemoglobinuria.

3. Novocain block of both lumbar sympathetic chains did not prevent the production of hemoglobinuria by ice packs.

4. For more than one month after resection of the left splanchnic nerves and removal of the first left lumbar ganglion, ice packs failed to cause hemoglobinuria; and after a similar operation on the right side, ice packs were ineffective for nearly six months.

5. Subsequent cervicodorsal ganglionectomy and complete lumbar ganglionectomy did not prevent the production of hemoglobinuria by ice packs.

6. The Donath and Landsteiner reaction for the presence of the characteristic hemolysin in the patient's blood serum was positive before the first operation but became negative after that operation and has remained so.

7. The patient's serum also contained auto-agglutinins in high titer, and these were not influenced by the operative procedures.

8. The operations have resulted in a great decrease in the frequency of attacks of hemoglobinuria following exposure to cold weather, but it is doubtful whether this effect will be permanent. Spinal anesthesia no longer prevents the induction of hemoglobinuria by ice packs.

9. The results of the investigation indicate that the sympathetic nervous system played a significant but not the primary rôle in the production of the attacks of hemoglobinuria.

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# ON THE NATURE OF THE SUBSTANCE(S) PRODUCING PAIN IN CONTRACTING SKELETAL MUSCLE: ITS BEARING ON THE PROBLEMS OF ANGINA PECTORIS AND INTERMITTENT CLAUDICATION<sup>1</sup>

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The mechanism of the production of pain in angina pectoris and intermittent claudication has been the subject of many studies during the last century and a half. Interest in this has recently been reawakened by the investigation of Lewis and his collaborators (1, 2). They showed that the continuous pain from contracting ischemic skeletal muscle of normal subjects is due not to vascular spasm but to the development by the contracting skeletal muscle of a factor producing pain, confirming a viewpoint proposed by the early workers in this field during the 18th century. In this laboratory we have recently been concerned with the nature of this factor producing pain. Our previous results (3, 4, 5) may be summarized as follows:

1. The factor producing pain accumulates in resting ischemic muscle as well as in contracting ischemic muscle, but at a much slower rate than in contracting muscle. The ratio between the two rates of accumulation is roughly proportional to the relative rates of energy exchange as indicated by  $O_2$  consumption and heat production (cf. Hill (6)).

2. The accumulation of the factor producing pain in contracting skeletal muscle which is not ischemic is accelerated (*a*) by stasis brought on by experimental venous engorgement, (*b*) by generalized anoxemia without stasis or ischemia, and (*c*) by increasing the rate at which the muscle is exercised.

3. After the exercise is stopped and the circulation to the muscle has been restored to normal, the factor producing pain takes a much longer time to disappear than does the pain. If the exercise is performed soon after a previous period of exercise or after a period of stasis or ischemia,

less exercise is required to cause pain in the subsequent test with the same muscle. This suggests a pre-pain state in which the amount of the factor producing pain while more than present normally is insufficient to stimulate the pain end organs.

These observations, when viewed in the light of the work of Lewis (2), MacWilliam and Webster (7), Reid (8) and others, suggested to us that the factor producing pain was a diffusible chemical product formed in the muscle during catabolism and disposed of in the presence of oxygen during the recovery of the muscle. The evidence for this contention was not conclusive, and the experiments in the present report were performed to test the hypothesis more completely. In addition, the experiments were planned to determine more precisely the nature of the factor producing pain.

## *1. The effect of varying the degree of circulatory stasis on the amount of exercise required to cause pain*

We have shown previously that circulatory stasis modifies the amount of exercise required to cause pain (4). In this study we attempted to analyze more quantitatively than hitherto the influence of the degree of circulatory stasis on the amount of exercise required to cause pain. This was necessary before we could investigate other phases of the problem.

*Method.* Four normal subjects were used in these experiments. All tests were carried out on the right arm with the subject in the prone position. The arm was supported at a right angle to the body, and the exercise consisted of clenching the hand around a special ergograph (c.f. Kissin (3)) 60 times per minute in time with a metronome. The cuff, which was placed around the upper arm, was suddenly inflated to the de-

<sup>1</sup> Aided by the Frederick K. Babson Fund for the Study of Diseases of the Heart and Circulation.

were carried out with the cuff pressure at 0 (no stasis), at 40, and at 80 mm. Hg and, to insure complete ischemia, at 140 mm. Hg. Measurements were made at a single cuff pressure but at different rates of exercise on each subject on a given day. This was repeated on different days at the other cuff pressures. Thirty to forty-five minutes of rest were allowed between exercises, and the order of the different rates of exercise was varied. Usually the effect of the exercises was studied at only one cuff pressure on any one day in a given subject.

At any given rate of exercise employed circulatory slowing produced an effect in the same direction as can be seen by comparing the vertical blocks at a given rate of exercise for different cuff pressures (Figures 2 and 3). The effect of circulatory slowing was less marked, however, at the faster rates than at the slower. This indicates that the circulatory slowing operates on the recovery phase of exercise, which is reduced in duration at the faster rates of exercise.

As anticipated, the effect of rate of exercise was less marked at the higher degrees of venous

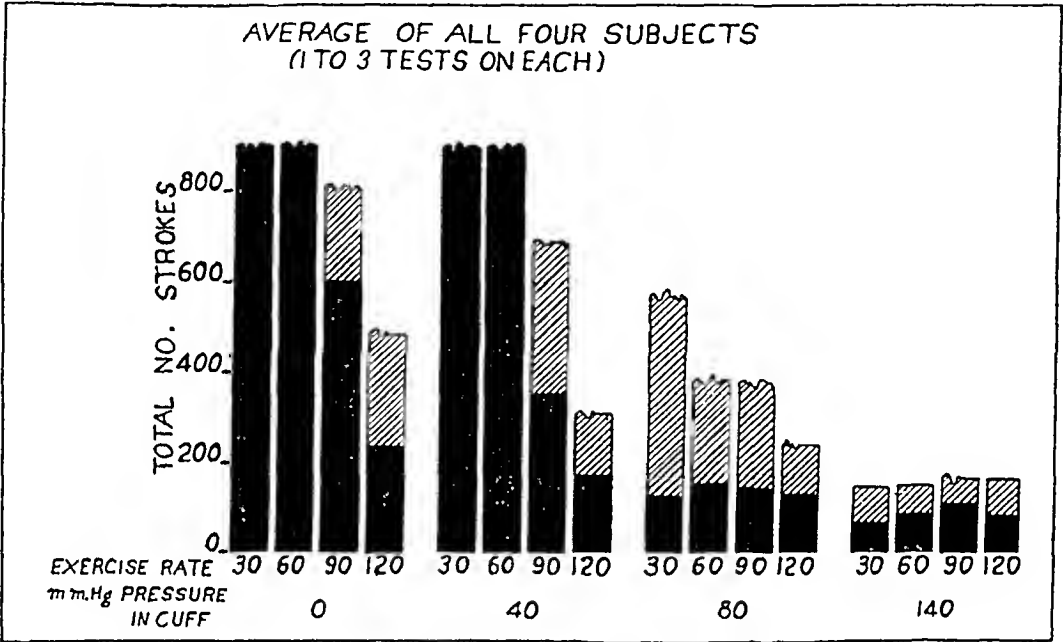


FIG. 2. GRAPHIC SUMMARY OF RESULTS ON FOUR SUBJECTS WITH VARYING RATE OF EXERCISE AT DIFFERENT DEGREES OF CIRCULATORY SLOWING.

The ordinates give the total number of strokes, the abscissae give the rate of exercise per minute (upper figures) and the pressure in the cuff around the upper arm at time of exercise (lower figures). Each group of four blocks is the average of all the tests carried out in all four subjects. Each of the four subjects was subjected to from 1 to 3 sets of tests, each set on a single day. The significance of the blocks is exemplified in Figure 1. A jagged line at the top of the vertical blocks at 900 denotes that the pain indicated did not develop by the time the exercise was stopped. A jagged line at the top of the vertical block below 900 denotes that the pain indicated did not develop because the exercise had to be stopped because of neuromuscular fatigue. A line at the top of the vertical block half jagged and half straight denotes that neuromuscular fatigue stopped about half of the tests and that pain of the type indicated stopped the other half. Discussed in text.

*Discussion of results.* Although the tests with different cuff pressures were carried out on different days instead of at one sitting, we confirmed the observation noted in the preceding section regarding the action of varying degrees of circulatory slowing on the onset of pain and added observations on the appearance of fatigue.

occlusion than when the circulation was free, and the effect disappeared when circulatory standstill was produced. This is most clearly shown in the onset of mild pain, but the tendency was present also in the onset of almost unbearable pain. Assuming that the exercise per stroke was constant at different rates, these results indicate that the

accumulated amount of the factor producing pain varies inversely as the time allowed for recovery. The time for recovery is less effective when the oxygen supply is limited, as in circulatory slowing, or when the oxygen supply is practically absent, as in circulatory standstill, than when the oxygen supply is adequate. Neuromuscular fatigue likewise appears after less exercise when the rate of exercise is faster, showing that it too depends on the time available for recovery from exercise.

quired to cause pain there than previously. Preliminary experiments convinced us that exercising one group of muscles temporarily alters the amount of exercise required to cause pain in other groups of muscles, even when precautions are taken to prevent the transport of substances from the former to the latter. The experimental procedure had to be elaborated, therefore, in order to take account of this complicating factor.

*Method.* The usual arm-exercise with the er-

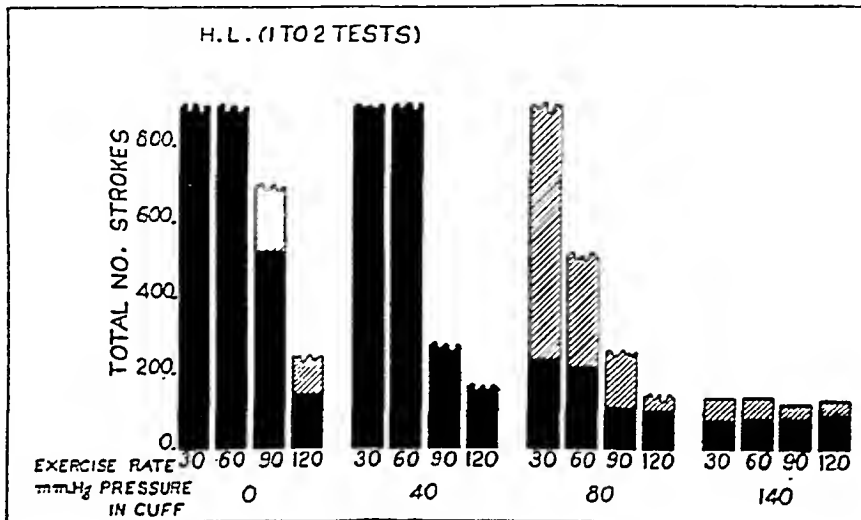


FIG. 3. GRAPHIC SUMMARY OF RESULTS IN A SINGLE TYPICAL SUBJECT WITH VARYING RATE OF EXERCISE AT DIFFERENT DEGREES OF CIRCULATORY STASIS.

The significance of the blocks etc. is as in Figure 2. The results are the average of the tests carried out. Discussion is in the text.

The application of these results to the problem of pain in angina pectoris would suggest that the rate of the heart beat would be more important when coronary circulation insufficiency was advanced, and that pain would appear in such a case with less work by the heart.

### III. Evidence indicating that the factor producing pain is a substance which diffuses in and out of the blood stream

No clear proof exists that the factor producing pain is a substance(s) which diffuses into and out of the blood stream. In principle, the proof of diffusion depends on finding that if one group of ischemic muscles undergoes exercise until pain appears, then if the blood from this limb has passed to another limb, less exercise will be re-

quired to cause pain there than previously. The graph was carried out in 17 sets of 4 tests on normal subjects at 60 strokes per minute. Rest intervals (30 to 45 minutes) were allowed after each exercise, the sequence being varied in the different sets except that (d) always followed (c) (*vide infra*). The total number of strokes necessary to bring on slight, moderate and almost unbearable pain was counted.

The four different conditions preceding the arm-exercise were as follows:

(a) Leg-exercise was provided on a friction-brake bicycle ergometer at the rate of 60 strokes per minute. The legs were compressed at the upper thigh by raising the pressure in the cuff approximately to 220 mm. Hg. Exercise was continued until moderately severe pain developed. The subject then walked to the bed preparatory to undertaking the arm-exercise. The pressure in

the cuffs on the legs was then released. Twenty seconds later, when blood from the leg had arrived in the arm, the cuff on the arm was inflated (160 mm. Hg) and the arm-exercise was begun. This we will refer to as *exercise after trapping*.

(b) For comparison, arm-exercise was carried out without previous leg-exercise, compression of the arm (160 mm. Hg) being simultaneous with the onset of exercise. This we will refer to as the *control for exercise after trapping*.

(c) In order to prevent the action of the blood coming from the exercised legs from affecting the exercising arm, the arm was compressed (160 mm. Hg) before and during the leg-exercise and kept so until the end of the arm-exercise. A pressure of 160 mm. Hg was found to be above the systolic pressure in the arm in all the subjects, even during the leg-exercise. This we will refer to as *exercise without trapping*. Continuous compression of the arm is required because blood cannot be shut off from all of the muscles of the leg and diffusible material could, therefore, be transported from the leg throughout the body.

(d) Since a preliminary period of ischemia lessens the amount of exercise required to cause pain (4) it was necessary to compare this effect with that in *exercise without trapping*. While resting in bed, the arm was kept compressed (160 mm. Hg) for 2 to 4 minutes, which was the time required for the onset of pain in the leg in the experiments on *exercise without trapping* and therefore the interval in these experiments during which the arm was compressed before starting arm-exercise. This we will refer to as the *control for exercise without trapping*. This exercise was carried out after the *exercise without trapping*.

*Discussion of results.* In order to ascertain the specific effect of blood from the exercised leg when trapped in the arm prior to exercise, free from other influences due to the leg-exercise, the following computations were made (cf. Table I).

Equation 1: (Onset of pain in *control for exercise without trapping*) minus (onset of pain in *exercise without trapping*) equals (onset of pain in arm due to factors following leg-exercise other than that mediated by the blood carried to the arm).

Equation 2: (Onset of pain in *control for exercise after trapping*) minus (onset of pain in *exercise*

TABLE I

*Calculations used in a typical set of exercises, also shown graphically in Figure 5, to show how the effect of the diffusible pain-producing substance(s) is determined.*

Subject C. H.			
Type of exercise	Onset of pain (number of strokes)		
	Slight	Moderate	Almost unbearable
<i>Control for exercise after trapping</i> .....	92	118	147
<i>Exercise after trapping</i> .....	86	119	137
A from Equation 1.....	-6	+1	-10
<i>Control for exercise without trapping</i> *.....	90	109	130
<i>Exercise without trapping</i> .....	125	160	196
B from Equation 2.....	+35	+51	+66
A minus B from Equation 3.....	-41	-50	-76

\* Since leg-exercise was carried out for 2 minutes to produce pain in the legs in this test, the preliminary compression of the arm in this control was carried out for the same period before beginning the arm-exercise.

*cise after trapping*) equals (onset of pain in arm due to all factors following leg-exercise).

Equation 3: (Onset of pain in arm due to all factors following leg-exercise (from Equation 2)) minus (onset of pain in arm due to factors following leg-exercise other than that mediated by the blood carried to the arm (from Equation 1)) equals (onset of pain in arm due to factor following leg-exercise mediated by the blood carried to the arm).

Columns I in Figure 4 show the scatter of results depicting the effect of factors following leg-exercise other than that mediated by the blood carried to the arm to be exercised, on the onset of slight, moderate and almost unbearable pain in the subsequent arm-exercise. Columns II of Figure 4 show the scatter of results depicting the combined effect of all factors following leg-exercise on the onset of the various degrees of pain in the subsequent arm-exercise. Columns III of Figure 4, derived in each set of exercises by subtracting algebraically the value in Column II from that in Column I, show the scatter of results depicting the effect of the factor following leg-exercise mediated by the blood carried from

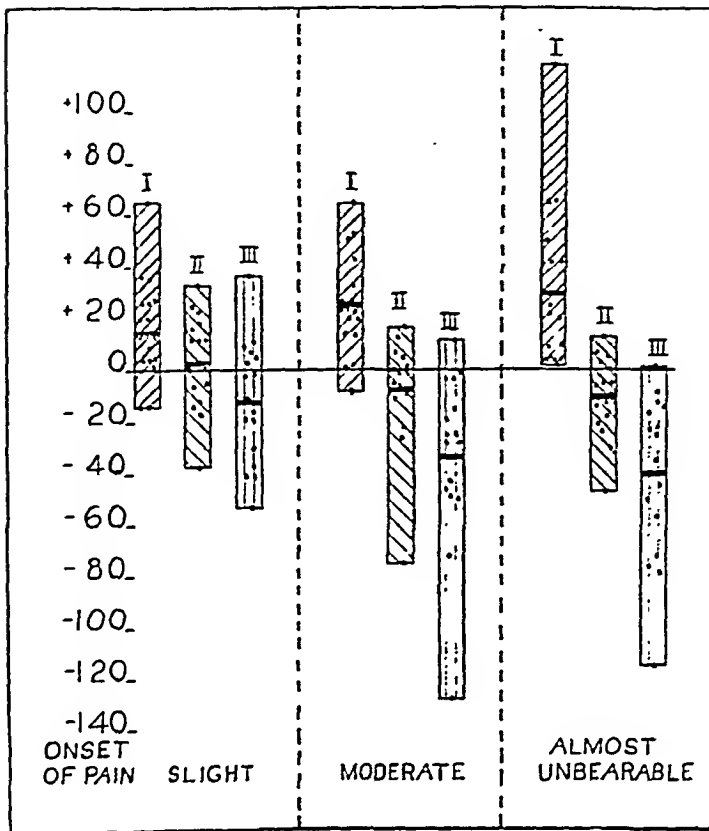


FIG. 4. GRAPHIC SUMMARY OF RESULTS IN 17 SETS OF TESTS ON 12 NORMAL SUBJECTS, SHOWING THE EFFECT ON THE AMOUNT OF EXERCISE REQUIRED TO CAUSE PAIN IN *Exercise without Trapping* (COLUMN I), IN *Exercise after Trapping* (COLUMN II), AS WELL AS THE COMPUTED EFFECT OF THE PAIN-PRODUCING SUBSTANCE(S) DIFFUSING INTO THE BLOOD FROM THE EXERCISING LEGS AND DIFFUSING OUT INTO THE MUSCLES OF THE ARM WHEN THE BLOOD FROM THE LEGS IS TRAPPED IN THE ARM TO BE EXERCISED (COLUMN III).

The columns are grouped for the onset of slight, moderate and almost unbearable pain. The ordinates show the effects in terms of total number of strokes; — means a decrease, and +, an increase in the number of strokes required to cause pain as compared with the controls for the subject. The manner of calculating each of these columns is given in the text: viz. Column I is determined by Equation 1; Column II by Equation 2; and Column III by Equation 3. Each column gives the range of scatter of the results in the 17 subjects. The results of the individual experiments are shown by the dots within the columns and the arithmetic average of the 17 tests in each column is shown by a horizontal line. Discussed in text.

the leg and trapped in the arm on the onset of various degrees of pain in the subsequent arm-exercise. The arithmetical average of each of these columns is shown by the horizontal heavy line within each column, and inspection will show that this is close to the median.

This figure, and also the summary of the four typical sets of exercise depicted in Figure 5, shows that exercising the legs to the point of moderately severe pain has a two-fold effect on the subsequent development of pain in the arm-exercise, most clearly seen with the severer de-

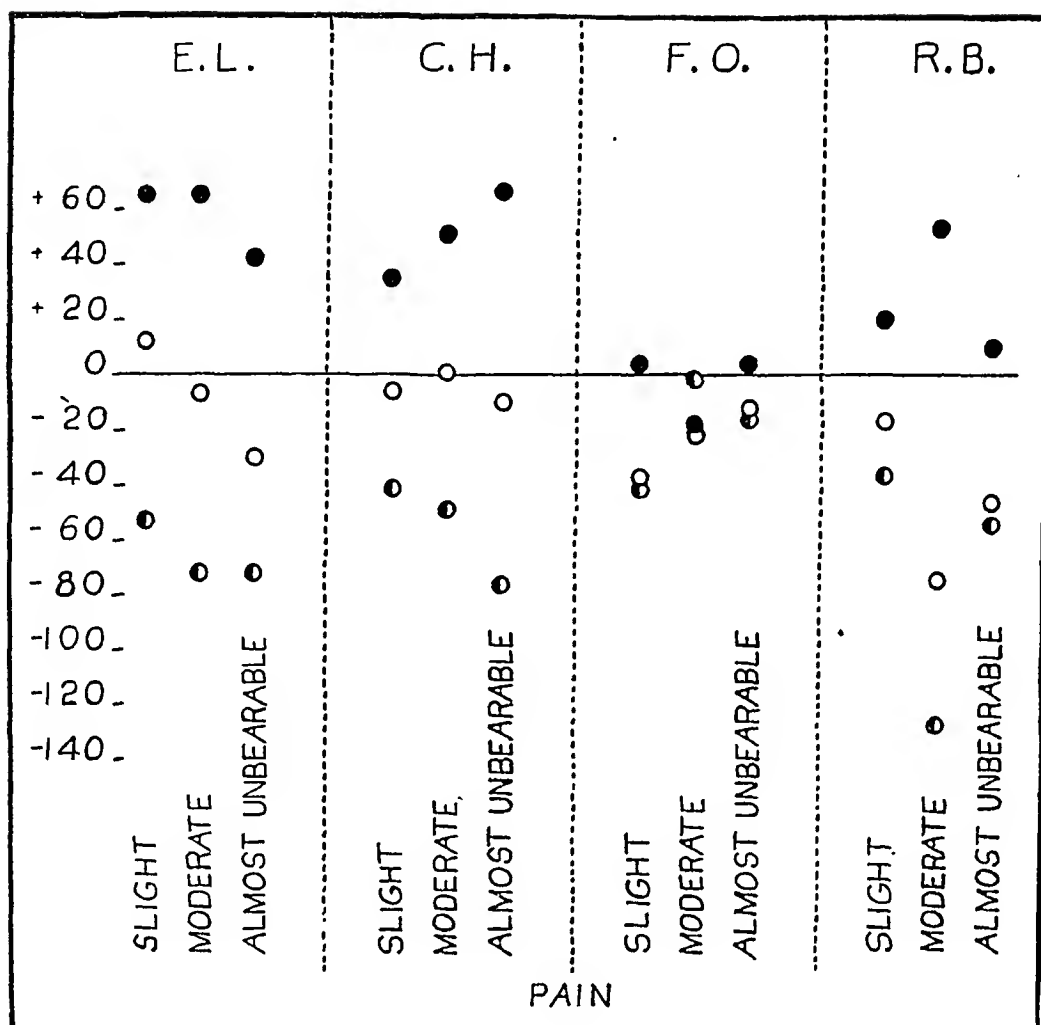


FIG. 5. GRAPHIC SUMMARY OF RESULTS IN 4 SETS OF TESTS, TYPICAL OF ALL, SHOWING THE EFFECT ON THE AMOUNT OF EXERCISE REQUIRED TO CAUSE PAIN IN *Exercise without Trapping* (SOLID CIRCLES), IN *Exercise after Trapping* (OPEN CIRCLES), AS WELL AS THE COMPUTED EFFECT OF THE PAIN-PRODUCING SUBSTANCE(S) DIFFUSING INTO THE BLOOD FROM THE EXERCISING LEGS AND DIFFUSING OUT INTO THE MUSCLE OF THE ARM WHEN THE BLOOD FROM THE LEGS WAS TRAPPED IN THE ARM TO BE EXERCISED (HALF SOLID, HALF OPEN CIRCLES).

The calculations are carried out in accordance with Equations 1, 2 and 3 in the text. The ordinates show the effect in terms of total number of strokes; — means a decrease, and +, an increase in the number of strokes required to cause pain as compared with the control for the subject. Discussed in the text.

degrees of pain in the arm. One action, the one not carried by way of the blood to the arm, tends to increase the amount of exercise required to cause pain, and the other action, that mediated by something transported by way of the blood to the arm, tends to decrease the amount of exercise required to cause pain. These actions do not appear clearly in every experiment but, considering the variables involved in such testing, these tendencies, it seems to us, are quite striking and surprisingly constant in the entire series. Considering the magnitude

of the changes observed in most experiments, especially in the onset of the severer forms of pain, the facts as stated above seem to be beyond peradventure.

The manner in which the *exercise without trapping* augments the amount of exercise required to cause pain in the arm is not known. Several possibilities suggest themselves. It is possible that the effect is due to a change in the sensorium for perception of pain brought about by the mediation of the blood passing to the brain from the exercising

legs. As mentioned above, it is impracticable to occlude the entire blood supply of the exercising legs. Hence diffusible materials from these parts, while kept out of the arm to be exercised, nevertheless can pass to other parts of the body, including the brain. The threshold of pain-perception may be raised in this way. A similarly blood-borne factor may affect the motor elements of the central nervous system so that the subsequent arm-exercise is carried out more efficiently as regards chemical change. Likewise an alteration may be produced by a reflex action either on the sensorium or motor elements of the central nervous system or on both. Still other possibilities exist which we are investigating. One thing, however, is certain, namely, that the augmentation of the amount of exercise required to cause pain in the *exercise without trapping* is not due to any blood-borne substance carried to the muscles to be exercised.

Making due allowance for this tendency to increase the amount of exercise required to cause pain, demonstrated in the *exercise without trapping*, in interpreting the results obtained in the *exercise after trapping*, brings out the action mediated by the blood on the muscles of the arm. This latter action is to decrease the amount of exercise required to cause pain. In other words, these results indicate that a substance(s) diffuses out of the exercising muscles of the leg into the blood stream, is carried by the blood stream through the lungs and to the muscles of the arm where it diffuses out and adds its effects to those of the substance(s) formed in the exercising arm. The net result is that less pain-producing substance(s) needs to be liberated by the exercising muscles of the arm to cause pain.

The fact that the blood exhibiting this additive effect is being carried to the arm past the lungs, would rule out the possibility that the pain-producing substance(s) was volatile, viz.  $\text{CO}_2$  or lack of  $\text{O}_2$ , since the former is eliminated from the blood and the latter is supplied to it before the blood reaches the arm. *It must, therefore, be some nonvolatile substance(s) liberated during muscular activity.*<sup>2</sup>

If, as our results indicate, the pain-producing substance(s) is diffusible in and out of the blood stream, then it is probable that an attack of angina pectoris can be precipitated not only by the accumulation of pain-producing substance(s) liberated by the heart during its contraction but also in part by the transport to the heart of such pain producing substance(s) from other parts of the body. Thus, during exercise the pain producing substance(s) liberated from the skeletal muscles, and in states of relative anoxemia from all parts of the body, pours into the blood stream and diffuses into the heart where it is added to that liberated by the heart. Since it is well known that the chemical exchange during exercise, and to a lesser extent during anoxemia, is much more intense in the skeletal muscles than in the heart, this relationship is of practical importance. Thus, in angina of effort the attack may be due not only to the accumulation of pain-producing substance(s) liberated by the heart itself but to transport of this substance(s) to the heart from the exercising skeletal muscles.

#### *IV. Is the pain-producing substance(s) acid in character?*

The demonstration in the foregoing experiments that the pain-producing substance(s) is diffusible and that it is not  $\text{CO}_2$  or lack of  $\text{O}_2$  limits the possibilities to two groups of substances formed during exercise, viz. (1) acid-metabolites such as lactic acid and some of the phosphoric acids and (2) non-acid metabolites such as creatinine. It is not practicable to test out the effects of these substances in man, and it is not easy to judge the reaction to pain in animals following injections. However, we felt that we could approach the problem by studying the effect which

exercise. This possibility is extremely unlikely, however, in view of facts previously established by us and by others in connection with the factor producing pain and in view of the well known fact that the alterations in exercise are due to liberated substances and not to deficiencies of substances, unless one is inclined to press the point that the appearance of one substance is accompanied by the disappearance of another. The only substance known to be removed from the blood in appreciable quantities is  $\text{O}_2$ . This has been restored to the blood in our experiments during passage of the blood through the lungs.

<sup>2</sup> These results are consistent with the possibility that the action is not due to substances liberated by exercising muscle but to something removed from the blood during



the pain-producing substance(s) may be an acid metabolite, or at least one that operates by altering the acid-base balance in and around the end-organs for pain, they should not be considered as in any sense final proof of this. Several other possibilities suggest themselves. The action of the sodium bicarbonate may be on the sensorium or on the motor elements of the central nervous system; in the former case, raising the threshold to pain perception, in the latter increasing the efficiency of the exercise in terms of amount of chemical change. It is unlikely that the reverse is true in the experiments with hypercapnia since the blood and tissues other than those of the exercising arm are probably restored to normal before pain appears, unless one assumes a long lag in the wearing off of the hypercapnic effects. A much more likely possibility is that if the pain-producing substance(s) is not acid in character then the  $\text{CO}_2$  and  $\text{NaHCO}_3$  alter the threshold of the endings for pain in the exercising arm and produce their effect in this manner. While these possibilities and probably others exist, we are strongly inclined to the view that the pain-producing substance is acid in character and propose this as a working hypothesis until evidence is advanced to disprove it. In fact, we are tempted to speculate, without warrant, that the substance may be lactic acid, the non-volatile acid formed in greatest amounts during exercise. It is interesting to note that Moore et al. (10) have found that acid substances, including lactic acid, when injected into the vessels of the limbs of animals produce responses indicating pain more easily than do other substances.

There is, in any event, no doubt that  $\text{CO}_2$  and  $\text{NaHCO}_3$  have an additive action with the pain producing substance(s); the  $\text{CO}_2$  acting in a positive, and the  $\text{NaHCO}_3$  in a negative manner. This action appears to be on the acid-base balance, in that the effect may be produced by altering the buffering capacity of the tissues, by altering the pH of the tissues, or in both ways.

The possibility of negative summation of the  $\text{NaHCO}_3$  with the pain-producing substance was tested out on patients with intermittent claudication and with angina pectoris in cooperation with Drs. Bohning, Gutman and Perlow. This experiment was suggested to us by Dr. Emanuel Lib-

man when discussing our results with him. The results will be published later. Suffice it to say that the ingestion of large quantities of  $\text{NaHCO}_3$  produced some relief of pain in patients with intermittent claudication and with angina pectoris; this relief was not obtained with placebos used for control. This inference was based both on an estimate of the patient's subjective reactions and on the effect of test exercises. The therapy was in no way a cure, but some relief seemed to be obtained in most of the subjects.

Our results with  $\text{NaHCO}_3$  in the exercise of the arm lends some support to the view expressed by Laplace and Crane (9) that in some instances the failure to develop anginal pain or pain in intermittent claudication may be due to the earlier onset of slight degrees of neuromuscular (and muscular) fatigue imposing a restriction on the work of the heart or muscles and so limiting the accumulation of the pain-producing substances(s). This may be one mode of action of  $\text{NaHCO}_3$ . For example, in F. O., Figure 7, the  $\text{NaHCO}_3$  caused so early an onset of neuromuscular fatigue in relation to the appearance of pain that the exercise was terminated by fatigue before any pain appeared.

#### *V. Does training modify the time of appearance of pain in exercising muscle?*

In the course of this study we had occasion to compare in the two arms the amount of exercise required to cause pain in seven normal subjects. All were right handed, except F. O. who was ambidextrous. The standard arm-exercise was carried out as in the previous tests at the rate of 60 per minute and with cuff pressure 160 mm. Hg. The exercise of the two arms was carried out within 30 to 45 minutes of each other.

The results are shown graphically in Figure 8 and are in accord with those reported by Laplace and Crane (9). The left arm in all instances required less exercise to cause pain and neuromuscular fatigue than did the right. The reason for reporting this observation here is that it may have an important bearing on intermittent claudication and angina pectoris. The difference in the two arms is undoubtedly associated with training, since clenching of the hand had been carried out much

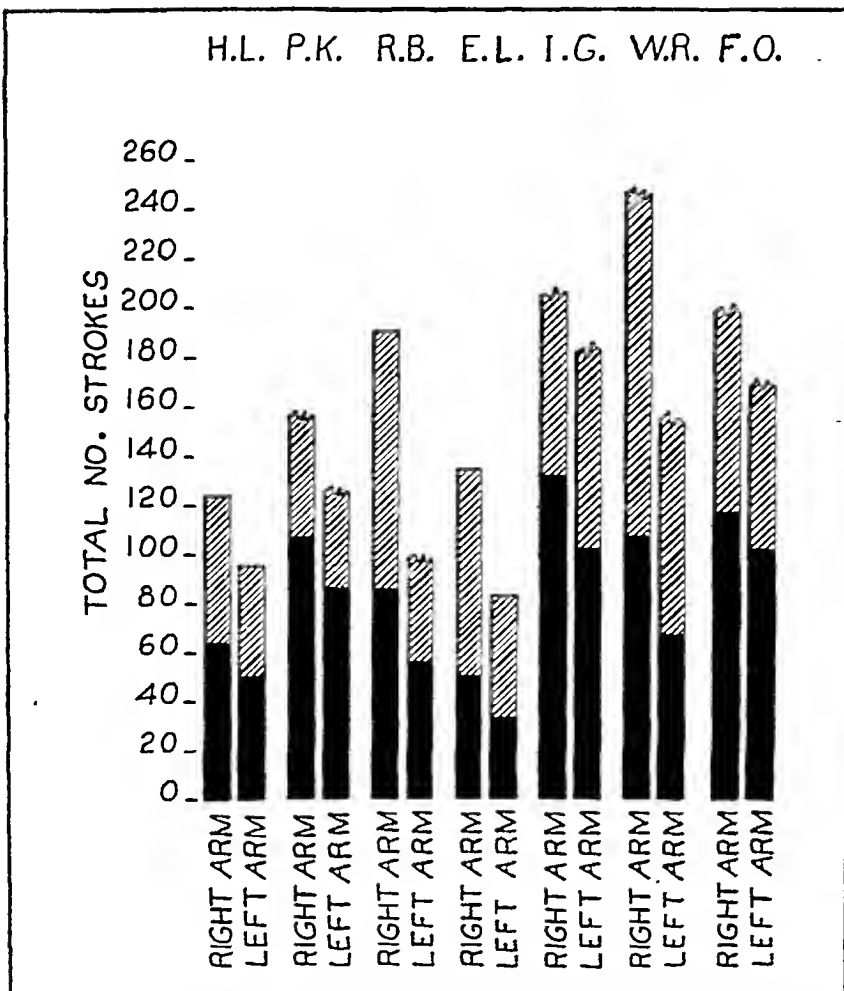


FIG. 8. GRAPHIC SUMMARY OF RESULTS IN SEVEN NORMAL SUBJECTS SHOWING THE DIFFERENCE IN THE AMOUNT OF EXERCISE REQUIRED TO CAUSE PAIN IN THE RIGHT AND LEFT ARMS.

Exercise was carried out as in experiments shown in Figure 1; exercise was at a rate of 60 contractions per minute and the cuff pressure was at 160 mm. Hg. The exercises of the two arms were carried out within 30 to 45 minutes of each other. The significance of the blocks etc. is as in Figure 1. Discussed in text.

more frequently with the right hand than with the left in all of these subjects.

The manner in which this difference in onset of pain and fatigue is brought about is not entirely clear. Several possibilities exist. The difference may be due to a difference in the threshold of the end-organs for pain and of the myoneural junctions and effector nerve endings, respectively, or to the difference in mechanical efficiency with which the trained right arm and untrained left do their exercises. Another possibility suggested by

our results with hypercapnia and ingestion of  $\text{NaHCO}_3$  is that the difference lies in the buffering capacity of the two muscles. There is evidence to suggest that the buffering power of muscles is increased with training (11).

The importance of avoiding unnecessary rest in patients subject to intermittent claudication or to anginal pain is evident from these results, and the importance of training in such patients is obvious. There is no doubt that if training is cautiously carried out the patient will be better able

to meet some of the demands which arise unavoidably in emergencies even when activity is limited.

#### SUMMARY

1. The main findings in this study with regard to the amount of exercise required to cause pain and fatigue in the muscles of the arm of normal subjects are:

(a) Circulatory slowing caused a decrease in the amount of exercise required to cause pain, the effect being disproportionately greater at high degrees of circulatory slowing. The amount of blood trapped in the arm played an insignificant rôle. Circulatory slowing had its greatest effect at the fastest rates of exercise.

(b) Increasing the rate of exercise led to a decrease in the amount of exercise required to cause pain when the circulation to the limb was unobstructed. Slowing the circulation led to a diminution of this effect of rate of exercise. This effect of rate of exercise disappeared when the circulation in the limb was stopped.

(c) Exercise of a large group of muscles of the leg to the point of pain had a two-fold action on the amount of exercise required to cause pain in a subsequent arm-exercise; viz., (1) by an action on the central nervous system it augmented the amount of exercise required to cause pain and (2) by an action through transport of blood from the exercised legs to the muscles of the arm it decreased the amount of exercise required to cause pain. Special procedures were required to separate these two effects.

(d) Increasing the  $\text{CO}_2$  content of the blood in the arm decreased the amount of exercise required to cause pain. Ingestion of large amounts of sodium bicarbonate increased the amount of exercise required to cause pain. Ingestion of sodium bicarbonate also tended to alleviate the pain of patients with intermittent claudication and of those with angina pectoris.

(e) In right handed subjects less exercise was required to cause pain in the left than in the right arm.

(f) The effects of these procedures on neuromuscular fatigue were not related quantitatively to their effects on pain. With certain procedures, such as the ingestion of sodium bicarbonate, the effects were opposite.

2. The significance of these results on pain and fatigue in muscle is discussed in the body of the paper. The salient conclusions from these experiments are:

(a) The time allowed for recovery between contractions in a rhythmically contracting muscle alters the rate of accumulation of the substance(s) leading to pain, implying that the pain-producing substance(s) is a product of metabolic muscular activity.

(b) The substance(s) causing pain diffuses into and out of the blood stream. It is non-volatile since it operates even after passing through the lungs.

(c) The appearance of pain in muscle is dependent not only upon the local production of pain-producing substance(s) but to a certain extent upon the transport of such a substance(s) from other regions.

(d) This non-volatile pain-producing substance(s) appears to be acid in character; at least its action is facilitated by acid and retarded by alkaline substances.

(e) Acids and bases exhibit summation of effect with the pain-producing substance(s) by changing the pH of the end organs, by altering their buffering capacity, or in both ways.

(f) Training tends to lessen the action of the pain-producing substance(s), probably by altering the buffering capacity of the muscle concerned.

(g) The variability in the appearance of fatigue, which is independent of pain, plays an important rôle in forestalling the appearance of pain under certain circumstances.

3. The bearing of these findings on the appearance of pain in angina pectoris and in intermittent claudication is discussed at the end of each section.

We are grateful to the volunteers, members of the Department, who undertook these exercises for us.

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# FURTHER OBSERVATIONS UPON THE CHANGES IN THE ELECTROLYTES OF THE URINE FOLLOWING THE INJECTION OF PARATHYROID EXTRACT<sup>1</sup>

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From previous studies (1, 2, 3, 4, 5) it now seems clear that one of the most striking changes found in the urine after the injection of parathyroid extract is the immediate increase in the absolute amount and in the concentration of inorganic phosphate. Goadby and Stacey (6) have also emphasized the increased excretion of phosphate after the administration of parathyroid extract. In agreement with our experience, these observers did not find at any time an increase in the level of inorganic phosphorus in the plasma, but occasionally noted a fall of that level after the excretion of increased amounts of inorganic phosphate in the urine.

They were unable to prevent the rise in excretion of urinary phosphorus by lowering the level of inorganic phosphate in the plasma with glucose administered at the time of the parathormone injection. The work of Goadby and Stacey, together with ours, serves to direct attention to the behavior of the kidneys in response to parathyroid extract.

In 1934, while studying the phosphate excretion by the kidney, one of us (5) observed that, when urine specimens were taken hourly before and after the administration of parathyroid extract, the pH showed a tendency to shift definitely to the alkaline side during the first hours after the extract was given. In order to examine this effect further it seemed advisable, although the bicarbonate of urine may be roughly estimated from the pH, to determine accurately the bicarbonate content of the urine before and after giving parathyroid extract and to compare the magnitude of any change found with that of the inorganic phosphate.

At the same time estimations of pH, sodium,

potassium, ammonium, phosphate, and chloride ions were made. As previous estimations have shown no significant alteration in urinary calcium, in experiments of the duration of the present ones, urinary calcium determinations were not done.

## EXPERIMENTAL

The subjects of the experiment were four male patients; three (G., S., K.) were convalescent from respiratory infections and ready for discharge from the hospital, the fourth, M., was a patient who had been operated upon for hyperthyroidism and who had developed hypoparathyroidism after operation. All patients were fasted and kept in bed for 12 hours before and throughout the duration of the experiment. Each patient was given 100 cc. of water by mouth each hour. Urine specimens in the three convalescent subjects were collected under oil, those of M. were not. The specimens were collected hourly for three hours before and four hours after the intravenous injection of 4 cc. of parathyroid extract (Lilly).

The analyses were made by standard methods as follows: pH by the quinhydrone electrode (13); CO<sub>2</sub> content by the Van Slyke method (14); chloride by the Volhard-Harvey titration (15); ammonium by the Van Slyke and Cullen method (16); potassium by the Kerr modification of the Kramer-Tisdall method (17); phosphorus by the Fiske and Subbarow method (18); sodium by the Butler and Tuthill application of the method of Barber and Kolthoff (19).

## RESULTS

In 1931, Albright, Bauer and Aub (7) made observations of the total acid-base balance in an individual receiving parathyroid extract. Their studies were made in three-day periods and their data include intake and output balances of many

<sup>1</sup> This work was aided by a grant from the council on Pharmacy and Chemistry of the American Medical Association.

of the electrolytes. They found an increase in water, chloride and total base of the urine during the periods of parathormone administration, as well as a decrease in the titratable acidity of the urine. In the periods studied by them, however, they concluded that the fluctuations, other than those of calcium and phosphorus, were probably not great enough to be "fundamental," although the decrease in water, total base and chloride excretion upon cessation of the administration of

stitute two of the principal points which we wished to study. Also they conducted their experiments in three-day periods, whereas in the present experiments the urine specimens were collected hourly. Our results are shown in Table I. It will perhaps be advisable to discuss separately below, the various observations.

*Water.* In all four subjects there was a distinct diuresis during the first and second hours after the injection of parathormone. This is a

TABLE I  
*Urinary constituents in hourly specimens*

Subject*	Volume				Total inorganic phosphate ‡												pH			
	G.	S.	K.	M.	G.	S.	K.	M.	G.	S.	K.	M.	G.	S.	K.	M.	G.	S.	K.	M.
Hourly specimen																				
	cc.	cc.	cc.	cc.	mgm.	mgm.	mgm.	mgm.	mM.	mM.	mM.	mM.	m.eq.	m.eq.	m.eq.	m.eq.				
1	105	78	77	100	23.8	12.2	21.0	52.5	0.76	0.39	0.54	1.4	0.8	0.6	0.9	2.2	5.5	6.8	6.4	6.4
2	125	128	68	100	32.7	12.9	30.2	50.5	1.05	0.41	0.78	1.3	1.2	0.6	1.2	2.5	5.8	6.8	6.1	6.9
3	122	120	163	70	39.4	9.2	57.6	35.0	1.26	0.29	1.47	0.9	1.5	0.5	2.4	1.9	6.0	7.1	6.4	7.2
Parathyroid extract 4 cc. i.v.																				
4	235	185	250	150	84.7	44.0	88.2	100.2	2.72	1.40	2.25	2.6	4.6	1.9	5.1	6.2	7.1	7.2†	7.4	7.9
5	185	112	140	100	80.9	60.0	138.5	100.3	2.61	1.90	3.55	2.6	4.3	3.2	7.8	6.2	7.0	7.3	7.3	8.1
6	50	70	39	50	66.0	46.7	78.0	74.8	2.14	1.50	2.00	1.9	3.8	2.5	3.5	4.6	7.4	7.0	6.7	7.8
7	50	28	50	15	68.4		74.0	30.9	2.20		1.90	0.8	4.0		2.7	1.9		6.6	5.9	

Subject	Total chloride				Total CO <sub>2</sub>			Total HCO <sub>3</sub> <sup>-</sup> ¶			Total Na			Total K		Total NH <sub>4</sub>			Titratable acid to pH 8.0
	G.	S.	K.	M.	G.	S.	K.	G.	S.	K.	G.	K.	M.	G.	K.	G.	K.	M.	
Hourly specimen																			
	m.eq.	m.eq.	m.eq.	m.eq.	mM.	mM.	mM.	m.eq.	m.eq.	m.eq.	m.eq.	m.eq.	m.eq.	m.eq.	m.eq.	m.eq.	m.eq.	m.eq.	cc. N/10
1	15.2	10.8	12.6	11.8	0.10	0.28	1.0	0.02	0.24	0.67	9.8	8.6	12.1	3.4	6.0	1.5	0.8	2.6	5.5
2	18.5	13.9	10.1	12.2	0.17	0.58	0.3	0.06	0.49	0.15	12.2	7.7	10.2	5.0	4.1	1.1	1.0	1.5	11.7
3	20.5	12.4	11.8	11.4	0.20	0.99	0.8	0.09	0.90	0.53	15.2	9.0	7.2	5.6	5.9	1.0	1.1	1.0	14.0
Parathyroid extract 4 cc. i.v.																			
4	21.3	11.6	18.7	17.1	6.00	1.70	8.0	5.40	1.60	7.60	20.4	18.0	21.3	11.5	12.7	0.1	0.6	0.4	7.1
5	9.0	10.8	11.4	8.5	3.10	2.00	4.8	2.70	1.90	4.50	9.8	12.8	14.7	7.4	10.3	1.0	0.9	0.1	2.0
6	5.0	8.6	4.0	4.4	0.80	0.60	0.6	0.76	0.50	0.48	5.7	5.4	8.1	5.4	3.4	0.5	0.6	0.4	13.4
7			4.9				0.1			0.04					3.5		1.3		27.0

\* No provision made in Patient M. for preventing escape of CO<sub>2</sub> from urine specimens.  
† This value not determined; approximate value estimated from total H<sub>2</sub>CO<sub>3</sub> and average free CO<sub>2</sub> of 1.9 mM. per liter.  
‡ M. eq. of total inorganic phosphate calculated from mM. of total inorganic phosphate and determined pH values.  
¶ HCO<sub>3</sub><sup>-</sup> calculated from pH and total CO<sub>2</sub> (HCO<sub>3</sub><sup>-</sup> + H<sub>2</sub>CO<sub>3</sub>) using chart from Peters and Van Slyke (12).

parathyroid extract was sufficient to cause them to comment upon the fact. Our experiments differ very distinctly from theirs in several ways. Albright, Bauer and Aub did not determine the bicarbonate or the pH of the urine, which con-

well-known effect. It does not occur invariably by any means (5), but is very often found. The period of diuresis in the four subjects was followed by a period of decreased urine volume.  
*Inorganic phosphorus.* As in previous experi-

ence, a prompt and very definite increase in the excretion of inorganic phosphate followed the injection of the hormone. This is shown in the table in milligrams, millimoles and in milliequivalents per hour. The increase in the 4th, 5th, and 6th hours of the experiment is shown clearly in all forms of recording. As the urine becomes more alkaline the ratio of  $\text{HPO}_4$  to  $\text{H}_2\text{PO}_4$  increases so that, when expressed in milliequivalents, the increase in phosphate excretion appears most striking. In the four experiments, although a diuresis occurred at the same time, the increase in inorganic phosphate was sufficient in nearly all specimens, taken after the extract was given, to produce an increase in the concentration as well as total amount. In former experiments (5), it was shown that even when the urine volume was smaller before the extract was injected than afterwards there was still a great increase in the excretion of phosphorus. This excretion is apparently not simply a washing out of phosphate in the process of diuresis.

*pH.* In three of the four subjects, there was a very distinct shift of the urine pH to a more alkaline range following the injection of parathormone. In the fourth subject, S., before the extract was given, the pH had risen to 7.1. The urine became slightly, but not remarkably, more alkaline following the administration of the extract. Some unpublished data on individuals with alkaline urine show that when the urine is already alkaline ( $\text{pH} > 7.2$ ) it does not usually become more alkaline after the injection of parathyroid extract. In Subject M. no provision was made to prevent the escape of  $\text{CO}_2$  from the urine samples. Loss of  $\text{CO}_2$  probably accounts in part for the very unusual alkalinity obtained (8).

*Bicarbonate.* Since it has been shown (8, 9) that alkaline urine contains more bicarbonate than acid urine it would perhaps be anticipated, from the pH changes, that the bicarbonate of the urine would be increased after the injection of parathyroid extract. The bicarbonate of the urine in the three individuals in whom it was studied showed a marked rise particularly in Subjects G. and K. The increase lasted two hours in both of these. The decrease in the third hour following the injection of extract was as abrupt as the rise had been in the first and second hours. When

the magnitude in milliequivalents of the bicarbonate excreted is compared with that of phosphate the two are seen to be of the same order. If the increase in excretion of bicarbonate should be found to endure characteristically for two hours, it might be said that the phosphate increase lasted longer. In the four experiments of this report the increase of phosphorus excreted in the urine lasted at least three hours. In other experiments (4) it has lasted five hours or more. The fact remains, however, that during the two hours following the injection of parathormone the excretion of bicarbonate rose and its total quantity in milliequivalents per hour was comparable to that of inorganic phosphate.

*Chlorides.* The alterations in chloride values in the urine after the injection of parathormone were somewhat indefinite. In the first hour after the injection, in two subjects there was no change; in two, a definite increase over the control hours. In the second hour after the injection all the values were lower than those for the first hour, whereas in three of the four the values for the second hour after the injection of extract were lower than the control values. This decrease in chloride excretion in the second hour following injection was observed in the cases formerly reported (5). Although the chlorides at first glance appear to follow the volume of urine, there is actually considerable variation in the concentration of chloride in the various specimens.

*Sodium and potassium.* The three subjects in whom observations were made all showed a definite increase in the output of sodium after the injection of parathormone. This was mainly observed in the first and second hours after the administration of the extract. The excretion of potassium (two subjects observed) also increased, but somewhat less markedly than that of the sodium. The concentration of sodium, as well as the total amount, underwent considerable change. The amount of potassium excreted seemed to vary fairly closely with the volume of urine.

*Ammonium.* In the three subjects studied for the ammonium ions, the excretion in the three hours following the injection of parathyroid extract was always somewhat less than in the control hours. In no specimen did it rise above the control level. There was certainly no evidence



of increased ammonia production by the kidney during the experiments.

When the sum of the cations, ammonium, sodium and potassium, in milliequivalents in any specimen is compared with the sum of the anions, the primary and secondary phosphate, bicarbonate and chloride, it is seen (Table II) that (except

TABLE II

*Sums of principal anions and cations excreted per hour*

Hourly specimens	Na <sup>+</sup> + K <sup>+</sup> + NH <sub>4</sub> <sup>+</sup> M. eq. total		HPO <sub>4</sub> <sup>-</sup> + H <sub>2</sub> PO <sub>4</sub> <sup>-</sup> + CL <sup>-</sup> + HCO <sub>3</sub> <sup>-</sup> M. eq. total	
	G.	K.	G.	K.
Subject				
1	14.7	15.4	16.0	14.2
2	18.3	12.8	19.1	11.5
3	21.8	16.0	22.1	14.7
	Parathyroid extract 4 cc. i.v.			
4	32.0	31.3	31.8	31.4
5	18.2	24.0	16.0	23.7
6	11.6	9.4	9.6	8.0

for G., hour 5, where the difference is 2.2 m.eq.) the difference of the sums is less than 2.0 m.eq. Without the sulphate and organic acids, magnesium and calcium figures (which are all in the normal individual less than 1 m.eq. per hour) no deductions may be made, but the comparison of the above sums shows that the principal anions and cations balanced roughly.

#### DISCUSSION

Without drawing definite conclusions from the data at hand, certain lines of thought are suggested and some points may bear emphasis. Following the injection of the parathyroid hormone there was a tendency for an acid urine to change abruptly to a more alkaline range. This change in pH was sometimes as much as 1.0 or even more. Accompanying this there was a great excretion of bicarbonate for two hours, such as is commonly found in alkaline urines. The excretion of inorganic phosphate was, as before observed, very much increased after the injection of parathormone. With the change in pH relatively more secondary phosphate ions were excreted, and the amount of phosphate in neutralizing (or base-matching) milliequivalents was very much increased after the injection of the hor-

mone. The chlorides showed a rather indefinite trend. Following the injection of the hormone large quantities of base were excreted, particularly in the first hour, when the chloride excretion was the same or higher, the phosphate and bicarbonate much higher, than in the control hours. The total base was sufficiently in excess of the acid radicles, of course, to make the urine alkaline.

As it has been shown before that calcium excretion does not increase in the first hours after the injection of parathyroid extract (and probably magnesium does not), the increased base excreted might have been any of the ions, sodium, potassium or ammonium. In the present experiments the ammonium ions certainly did not increase. The potassium increased moderately, roughly corresponding to the water, while the sodium showed in one instance a definite increase; in two instances, a large increase.

The foregoing observations give rise to two suggestions regarding the behavior of the kidneys after the injection of parathyroid extract. The first is that increased amounts of acid might be *primarily* excreted as a result of the action of the hormone. It has already been suggested by one of us (4) that the renal threshold for phosphate might be lowered by the hormone. The present experiments indicate that if the renal threshold is lowered for phosphate, it must also be lowered correspondingly for bicarbonate. Against the suggestion that the parathyroid hormone causes a primary increase in the excretion of phosphate and bicarbonate are two points. One of these is that the primary excretion of acid usually results in an acid urine, whereas the urine specimens containing the increased amount of phosphate and bicarbonate in the present instance were alkaline. In fact, the actual presence of large amounts of bicarbonate in the urine is very much against a primary acid excretion. The other point very much against it is that an increase in the excretion of acid is, as a rule, accompanied by an increased production of ammonia by the kidney. The ammonia production in the present experiments decreased, in fact, after the parathormone was given and during the hours when bicarbonate and phosphate were excreted in larger amounts.

The second suggestion that arises from the present and previous experiments is that admin-

istration of the parathyroid hormone might result in an increase *primarily* in the excretion of fixed base, particularly sodium, and that bicarbonate and phosphate accompany secondarily the increase in sodium excretion. This is a very tempting hypothesis, as it would explain the alkaline urine, the decrease in ammonia production, the bicarbonate and secondary phosphate excretion.

In view of the recent work of Loeb, Atchley, Benedict and Leland (10) and of Harrop, Soffer, Ellsworth and Trescher (11) showing that sodium is lost through the urine when the animal is deprived of adrenal cortical hormone, the suggestion might be made that the parathyroid hormone may have an effect opposite to that of the adrenal cortical hormone, in producing an increase in the output of base, particularly sodium, in the urine.

#### SUMMARY

1. The effect of the injection of parathyroid extract upon certain electrolytes of the urine was observed in four human subjects.

2. There was a tendency for the urine to become more alkaline after the injection of the extract.

3. There was not only an increase in the excretion of inorganic phosphate, as noted previously, but a marked excretion of bicarbonate as well, after the administration of the hormone.

4. There was a slight decrease in ammonium ions in the urine whereas the excretion of potassium and particularly of sodium ions was very much increased after the injection of parathormone.

5. The theoretical implications of these observations is briefly discussed.

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# IMMUNIZATION OF HUMAN SUBJECTS WITH THE SPECIFIC CARBOHYDRATES OF TYPE III AND THE RELATED TYPE VIII PNEUMOCOCCUS<sup>1</sup>

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Sugg and Harris and their respective coworkers were the first to study extensively the immunologic properties of a strain of pneumococcus closely related to, but not identical with, typical strains of Type III pneumococci (1, 2). The "Thomas" strain of these workers now can be classified definitely as a Type VIII pneumococcus, according to the classification of Cooper et al. (3). Both the Thomas strain and the Type VIII strains were shown, by these respective investigators, to produce in certain animal species antibodies of high titer against typical strains of Type III pneumococci. The titers were sometimes higher than the notoriously low homologous antibody titers obtainable by the injection of typical Type III strains. The reciprocal serological relationships between typical Type III strains and the related Type VIII strains were demonstrated with regard to passive protection in mice, agglutination, and precipitation. Antibody relationships similar to those described in the sera of lower animals were shown, by Finland and Winkler (4), to occur also in the sera of certain human subjects during convalescence from pneumonia due to these types. With regard to "natural antibodies," as measured by the pneumococcal power of the whole defibrinated blood or the protection afforded mice by the serum of normal human subjects, Finland and Sutliff found no such reciprocal relationship (5).

The specific carbohydrate from *Pneumococcus* Type VIII was recently isolated and its properties described by Brown (6). She found marked cross-precipitation between this carbohydrate and that of the Type III pneumococcus. In view of this finding and because of the poor antibody response of human subjects to injections of the

Type III polysaccharide (7), it seemed of interest to study the effect of injections of the Type VIII carbohydrate. This paper deals with the antibody responses to such injections and a comparison of them with the results of similar injections of different preparations of the specific Type III polysaccharide. Variations in the antibody response due to differences in dosage and in the route of injection are dealt with in a separate communication (8).

## EXPERIMENTAL

### *Subjects, materials and methods*

The Type VIII carbohydrate was obtained from Dr. Rachel Brown and was, in most respects, similar to the preparation which she described (6). Her analysis showed it to contain 0.72 per cent nitrogen, 0.11 per cent phosphorus, 39.69 per cent carbon, 6.15 per cent hydrogen, and 6.37 per cent ash. It yielded 69.5 per cent reducing sugars after acid hydrolysis. It precipitated the homologous type antiserum in a dilution of 1:4,000,000 and Type III antiserum in a dilution of 1:2,000,000. In the presence of the homologous immune rabbit serum, it fixed complement. Mice injected with amounts varying from 0.1 to 0.000001 mgm. were not protected against subsequent injections of culture. Most of the preparations of the Type III polysaccharide have been used in previous immunization studies (7, 9).

The subjects were adult hospital patients who were free from recent febrile diseases. Each individual received a single subcutaneous injection of 1.0 mgm. of one carbohydrate preparation over the deltoid insertion. This dosage and route were found to yield the best results with the Type VIII material (8). No local or general reactions occurred following any of the injections. Blood for the study of antibodies was drawn before and at intervals after injections. Phagocytosis was studied by a method similar to that used by Ward and Enders (10). Agglutinins were determined by the technique employed by Tillett and Francis (11), and the mouse protective titer was obtained by the method of Dochez (12). Organisms of maximum virulence were used throughout the experiments.

<sup>1</sup> This investigation was aided, in part, by a grant given in honor of Francis Weld Peabody by the Ella Sachs Plotz Foundation.

## RESULTS

*Homologous antibody response elicited by the Type VIII carbohydrate*

The results of tests for the development of agglutinins, mouse protective antibodies and opsonins for the homologous type of pneumococcus in the 12 subjects who received a single subcutaneous injection of 1.0 mgm. of the Type VIII carbohydrate are shown in Table I. They indicate that an appreciable titer of the antibodies was stimulated in each instance. The response was demonstrated with greater regularity in these subjects than in patients recovering from pneumonia due to the same type of organism. The maximum titer of antibodies was reached in about 2 weeks and was maintained, approximately at this level, throughout the period of observation in 4 subjects whose sera were studied 6 to 8 weeks, and in 2 subjects almost 5 months after the injection. In 1 subject (J. N.) a drop in titer occurred between the 49th and the 121st day, no blood having been obtained in this interval.

*Homologous antibody response to injection of Type III polysaccharide*

In previous attempts to immunize human subjects with Type III pneumococcus polysaccharide, single or multiple doses of 0.01 to 0.05 mgm. were given intracutaneously (7). In view of the more constant and higher grade response obtained by subcutaneous injections of 1.0 mgm. of the Type VIII carbohydrate, a study was made of the response of human subjects to Type III materials given in this manner. A single injection of 1.0 mgm. of one of 6 preparations of the Type III carbohydrate was given subcutaneously to each of 41 subjects, and the antibody response in them was studied in a manner similar to that employed for the Type VIII cases.

The results are summarized in Table II. In general, inconstant and low-grade responses were secured. They were similar to those previously obtained with the smaller doses given intracutaneously (7) and somewhat less marked than those observed to develop in patients recovering from

pneumonia due to the same type of pneumococcus (4).

TABLE I

*Antibody response to subcutaneous injection of 1.0 mgm. of Pneumococcus Type VIII soluble specific substance*

Subject Sex and age	Days after injection	Titer of Type VIII antibodies			Type III antibodies
		Agglu- tinins (serum dilution)	Mouse protection (Fatal doses per 0.2 cc.)	Phagocytosis (Average number diplococci per polymorpho- nuclear leukocyte)	
A.S. M 22	0	0	—	1.5	No change
	3	0	10	0.9	
	7	0	100	4.0	
	14	1:4	1,000,000	9.4	
	24	1:4	1,000,000	16.8	
	56	1:8	10,000,000	12.2	
F.C. M 45	0	0	0	2.0	No change
	2	0	10	2.9	
	7	0	1,000	6.2	
	14	1:2	1,000,000	—	
	31	1:4	100,000	18.8	
	50	1:4	100,000	11.6	
	146	0	100,000	13.1	
F.S. F 39	0	0	0	0.5	Protection: before = 0 14th day 1000 fatal doses 28th day 0 No other change
	3	0	—	1.4	
	7	1:2	1,000	21.1	
	14	1:16	1,000,000	11.5	
	28	1:8	1,000,000	21.8	
	47	1:8	1,000,000	11.6	
	143	1:8	100,000	31.8	
M.McG. F 35	0	0	100	2.8	Phagocytosis increased from 0.6 to 2.8
	3	0	10	1.4	
	7	1:2	10,000	21.4	
	14	1:8	10,000,000	10.4	
	63	1:16	1,000,000	19.2	
W.H.M. M 68	0	0	0	0.7	Protection: before = 10 fatal doses 25th and 44th day = 100 fatal doses
	8	0	10,000	3.3	
	15	1:2	100,000	11.8	
	18	1:4	—	9.8	
	25	1:4	100,000	16.4	
	44	1:4	1,000,000	16.0	
J.P.C. M 62	0	0	10	1.1	No change
	8	1:2	1,000	16.2	
	15	1:16	10,000	11.2	
	51	1:4	100,000	—	
S.B. F 59	0	0	0	5.8	No change
	7	0	0	7.6	
	14	1:2	0	26.8	
	19	1:2	100	38.2	
	28	1:8	100	30.0	
S.V.C. F 79	0	0	10	6.0	
	7	0	100	4.0	
	10	1:4	—	14.0	
	14	1:4	1,000,000	19.5	
J.N. M 40	0	0	0	2.5	No change
	49	1:8	100,000	7.7	
	121	1:2	10	2.8	
	131	1:2	100	1.3	
E.J.B. M 75	0	1:2	10	5.2	
	7	1:2	100	4.0	
	14	1:4	100,000	19.0	
M.K. F 62	0	0	0	2.0	No change
	3	0	10	1.6	
	7	0	10,000	19.6	
	11	32	1,000,000	13.0	
K.R. M 61	0	0	0	1.3	No change
	8	1:2	0	16.0	
	25	1:4	1,000	—	

TABLE II

*Antibodies against Type III and Type VIII pneumococci resulting from a single subcutaneous injection of 1.0 mgm. of the polysaccharides of these organisms*

Polysaccharide		Number of subjects	Number showing increases in titer of antibodies											
			Agglutinins*				Mouse protection†				Phagocytosis‡			
			0	2	4	8+	0	10	100	1000+	0 to 1	2 to 5	6 to 10	11+
Type	Lot													
Type VIII antibodies														
VIII	WS3	12	0	1	4	7	0	0	1	11	0	1	0	11
III	WS4c	8	7	1	0	0	5	2	1	0	5	3	0	0
III	WS3	6	6	0	0	0	4	2	0	0	4	1	0	1
III	WC	6	5	1	0	0	3	3	0	0	4	2	0	0
III	RS	5	2	1	1	1‡	3	1	0	1	2	2	0	1
III	FA	8	4	3	1	0	6	1	0	1	4	2	2	0
III	FB	7	5	0	2	0	4	8	0	0	3	1	1	2
All Type III lots		40	29	6	4	1	25	12	1	2	22	11	3	4
Type III antibodies														
VIII	WS3	12	12	0	0	0	10	1	0	1	11	1	0	0
III	WS4c	9	6	1	1	1‡	3	1	2	3‡	3	1	3	2‡
III	WS3	6	5	0	0	1**	3	2	0	1‡‡	5	1	0	0
III	WC	6	5	0	1	0	2	0	2	2‡‡	4	1	1	0
III	RS	5	3	1	0	1**	1	1	2	1	3	1	0	1
III	FA	8	6	0	1	1	2	4	0	2	6	1	1	0
III	FB	7	3	0	1	3††	2	1	1	3‡‡	3	1	1	2
All Type III lots		41	28	2	4	7	13	9	7	12	24	6	6	5

\* 0, 2, etc. = no increase, twofold increase, etc., in highest dilution showing agglutination.

† 0, 10, etc. = 0, tenfold, etc., increase in fatal doses of protection.

‡ 0 to 1, 2 to 5, etc., = increase in average number of diplococci phagocytosed per polymorphonuclear leukocyte. § 1: 32.

|| 1,000,000 fatal doses.

¶ Serum of same subject increased 16-fold in protection and an average of 22 diplococci per polymorphonuclear leukocyte.

\*\* 16-fold increase.

†† 1 increased 16-fold, another 32-fold.

‡‡ 10,000-fold increase.

§§ 2 increased 10,000-fold.

*Antibodies for the heterologous but related type following an injection of the Type III or the Type VIII pneumococcus carbohydrate*

The sera of all of the subjects receiving an injection of either the Type III or the Type VIII carbohydrate were tested for antibodies against both types of organism. The results are summarized in Table II. Only 1 of the subjects (F. S.) injected with the Type VIII material showed an appreciable development of antibodies

for the Type III organism. Other subjects, as described in the paper that follows (8), were encountered whose sera showed Type III antibodies in low titer following the injection of the Type VIII material in different amounts or by routes other than the subcutaneous one.

The Type III preparations used were all similar in their antigenicity as regards both the homologous and the related type of antibody. Antibodies of significant titer against Type VIII following injections of Type III polysaccharide were infrequent. Low titers of such antibodies, however, were demonstrated in almost half of the subjects who received Type III materials.

The findings described indicate that the common antigen of the Types III and VIII pneumococci is retained, at least in part, in these highly purified fractions. Less of it is retained in the Type VIII than in the Type III materials. In either case, however, the antibody response to the related type was less frequent than that following injections of the intact organisms in lower animals (1, 2, 3), or than that found in patients recovering from pneumonia due to these types (4).

*Development of antibodies against heterologous and unrelated pneumococcus types*

The development of antibodies for heterologous types of pneumococci has been reported to follow injections of Type I and II pneumococcus fractions in lower animals (13) and in human beings (14). In the present investigation, in addition to tests for antibodies against the homologous and the related types of organisms (Type III and Type VIII), similar tests were carried out on the sera of each subject with either Type I or Type II pneumococci. None of the subjects who received Type VIII injections showed any increase in antibodies against either of these types by any of the tests employed. Among those receiving the Type III polysaccharide, 3 subjects developed or increased their titer of antibodies against an heterologous type as shown by mouse protection and phagocytic tests. Agglutinins did not appear in the serum. Only 1 of the 3 subjects developed Type VIII antibodies.

One subject received 1.0 mgm. of Type III preparation FA and developed antibodies as follows: Type III ag-

glutinins appeared in dilution of 1:8, mouse protection against 10,000 fatal doses and an increase in phagocytic titer from 0.8 to 10.3 diplococci per polymorphonuclear leukocyte; Type VIII agglutinins increased from 1:2 to 1:8, protection from 10 to 10,000 fatal doses and the phagocytic titer from 0.5 to 7.0; Type I protection increased from 1000 to 100,000 fatal doses and the phagocytic titer from 13.4 to 23.5. In a second subject receiving the same material no Type III or VIII antibodies appeared, but the protective titer of the serum against Type I increased from 10 to 10,000 fatal doses and the phagocytic titer for this type increased from 1.9 to 22.4 diplococci per polymorphonuclear leukocyte. A third subject who received preparation RS developed protective antibodies against 100 fatal doses of Type III and against 1000 fatal doses of Type II pneumococci. The phagocytic titer increased only against Type II pneumococci, the titer being 1.7 before and 16.7 after the injection.

These findings indicate that a species antigen may be present in the pneumococcus but that it is largely lost in the process of preparation of the carbohydrate fractions (cf. 15). In the case of some of the Type III preparations, the data suggest that sufficient of this species antigen was retained to give rise to Type I or Type II antibodies in measurable amounts in an occasional subject.

#### SUMMARY

1. High titers of antibodies against the homologous type were demonstrated regularly in human subjects following the subcutaneous injection of 1.0 mgm. of a highly purified, type-specific carbohydrate of *Pneumococcus* Type VIII.

2. Various preparations of the Type III pneumococcus polysaccharide, given in the same manner, produced antibodies for the homologous type with less regularity and in lower titers.

3. Occasional subjects injected with Type III or with the related Type VIII pneumococcus carbohydrate developed antibodies against pneumococci of the heterologous but related type. Such cross-immunity, however, was less frequent and of a lower grade than that encountered either in animals immunized with the whole organism or in human patients recovering from pneumonia due to these types of pneumococcus.

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# THE INFLUENCE OF DOSAGE AND ROUTE OF INJECTION ON THE ANTIBODY RESPONSE OF HUMAN SUBJECTS TO THE SPECIFIC CARBOHYDRATE OF THE TYPE VIII PNEUMOCOCCUS<sup>1</sup>

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The readiness with which immune bodies could be demonstrated in human subjects following the injection of the specific carbohydrate of the Type VIII pneumococcus (1) offered an opportunity to study, in man, the effect of dosage and of the route of injection upon antibody production with a carbohydrate antigen. Although numerous investigators have made similar studies with whole bacterial antigens in lower animals and in man, no data are available concerning the comparative antigenic activity, in man, of purified bacterial fractions given in different amounts or by different routes. The development of antibodies following injections of type-specific polysaccharides was first demonstrated by Francis and Tillett (2) in the course of their investigations on cutaneous reactions in lobar pneumonia.

## EXPERIMENTAL

### *Subjects, materials and methods*

The subjects chosen for this investigation, the Type VIII pneumococcus specific carbohydrate used, and the methods employed for the antibody studies have been described in the previous communication (1). Except in the subjects included in that report, phagocytic tests were performed irregularly. Only the results of the tests for agglutinins and mouse protective antibodies, therefore, will be considered here.

The intracutaneous injections were made into the volar aspect of the forearm, the subcutaneous ones over the deltoid insertion, and the intravenous doses were given into the antecubital vein. Because antibodies were often stimulated by minute amounts of the carbohydrate given intracutaneously, special precautions were taken, in making the intravenous injections, to avoid getting any of the material into or under the skin. This was done by attaching the syringe containing the carbohydrate solution to the needle only after a free flow of blood was obtained, and then, following the injection, drawing some blood into the syringe before the needle was withdrawn. All of the injections were made with the carbohydrate in solution in physiological saline which was especially prepared

(3). The intracutaneous injections were made in 0.1 cc. amounts. All other injections were in 1.0 cc. amounts except the 5.0 mgm. doses, which were given in a volume of 1.25 cc. No local or general reactions followed any of the injections.

## RESULTS

In Table I, in the previous communication (1), there are tabulated the homologous type-specific antibody titers of each of the bloods obtained from subjects who received 1.0 mgm. of the Type VIII carbohydrate subcutaneously. The results of the studies of the individual sera in the remainder of the subjects now under consideration corresponded, in a general way, to those shown in that table. For the sake of brevity, and because the major interest in this paper lies in a comparison of the antigenic effects that variations in the dose and route of injection have upon the development of antibodies, only the antibody titers of the sera obtained before injection and the ones showing the maximum titers after injection in each subject will be noted. These titers in each of the individuals receiving various doses of the carbohydrates of the Type VIII pneumococcus by the intravenous, subcutaneous or intracutaneous route are shown in Table I. The maximum accretions in titer of agglutinins and mouse protective antibodies are represented graphically in Figures 1 and 2, respectively, in such a way as to make possible a comparison of the antibody response to each of the various doses. While the number of subjects in each group is small, the trends are apparent and worth noting.

The results of the studies, shown in the table and figures, may be summarized briefly: 1. Each of the doses tested, which ranged from 5.0 mgm. to 0.001 mgm. intravenously, from 1.0 mgm. to 0.001 mgm. subcutaneously, and from 0.15 mgm. to 0.0001 mgm. intracutaneously, stimulated in some subjects an appreciable titer of the homologous type-specific antibody. 2. In general, regardless of the route of injection, when decreasing

<sup>1</sup> This investigation was aided, in part, by a grant given in memory of Francis Weld Peabody by the Ella Sachs Plotz Foundation.



TABLE I

Homologous type antibody response to single injections of Type VIII soluble specific substance

Subject	Sex	Age	Amount injected	Agglutinins (serum dilution)		Mouse protection (fatal doses per 0.2 cc.)	
				Be-fore	After	Before	After
		years	mgm.				
A. Intravenous							
H.J.B.....	M	56	5.0	1:2	1:64	100	1,000,000
J.B.....	M	45	5.0	0	1:8	1,000	1,000,000
M.A.....	F	79	5.0	0	0	0	0
T.N.....	M	69	5.0	0	0	0	0
I.C.....	F	44	5.0	0	0	0	0
F.K.....	M	61	5.0	0	0	0	0
C.D.....	M	54	1.0	0	1:16	0	100,000
W.P.B.....	M	59	1.0	0	1:8	0	100,000
T.T.....	M	73	1.0	0	1:128	100	100,000
C.McF.....	F	29	1.0	0	1:4	0	100,000
D.B.....	F	65	1.0	0	1:16	10,000	1,000,000
D.L.....	M	71	1.0	0	1:4	0	1,000
T.R.....	M	24	1.0	0	1:4	0	1,000
A.C.....	F	73	1.0	0	0	0	10
F.B.....	M	50	0.1	0	1:64	0	100,000
C.B.....	M	59	0.1	0	1:2	0	100,000
G.M.....	M	56	0.1	0	1:2	0	100,000
J.M.....	M	51	0.1	0	1:16	1,000	1,000,000
H.S.....	M	49	0.1	0	1:4	0	10
M.L.....	M	58	0.1	0	0	0	0
J.P.....	M	46	0.01	0	1:8	0	1,000
C.C.....	M	72	0.01	0	0	0	1,000
I.G.....	M	76	0.01	0	1:4	100	1,000
T.F.....	M	13	0.01	0	0	0	0
E.B.....	M	24	0.01	1:4	1:4	100	100
J.K.....	M	59	0.01	0	0	1,000	100
C.Ca.....	M	21	0.001	0	1:4	0	1,000
E.L.....	M	25	0.001	0	1:2	10	10,000
V.B.....	M	45	0.001	0	0	0	0
C.M.....	M	37	0.001	1:4	1:2	10,000	10,000
F.T.....	M	39	0.001	0	0	0	0

B. Subcutaneous

M.K.....	F	62	1.0	0	1:32	0	1,000,000
A.S.....	M	22	1.0	0	1:8	10	10,000,000
F.S.....	F	39	1.0	0	1:16	0	1,000,000
M.McG.....	F	35	1.0	0	1:16	100	10,000,000
F.C.....	M	45	1.0	0	1:4	0	1,000,000
W.H.M.....	M	68	1.0	0	1:4	10	1,000,000
S.V.C.....	F	79	1.0	0	1:4	10	1,000,000
J.N.....	M	40	1.0	0	1:8	0	100,000
J.P.C.....	M	62	1.0	0	1:16	10	100,000
E.J.B.....	M	75	1.0	1:2	1:4	10	100,000
K.R.....	M	61	1.0	0	1:4	0	1,000
S.B.....	F	59	1.0	0	1:8	0	100
J.H.....	M	25	0.1	0	1:32	0	100,000
J.McC.....	M	52	0.1	0	0	0	100,000
W.C.....	M	51	0.1	0	1:4	0	10,000
F.M.....	M	14	0.1	0	0	0	1,000
C.B.....	F	39	0.1	0	0	0	100
F.W.....	F	24	0.1	0	0	10,000	100,000
E.R.....	M	32	0.01	0	1:4	0	100,000
J.D.S.....	M	68	0.01	0	1:2	0	100,000
A.G.....	M	32	0.01	0	1:4	0	1,000
J.G.....	M	49	0.01	1:4	1:32	100,000	1,000,000
W.H.....	F	60	0.01	0	0	10	1,000
M.P.....	F	50	0.01	1:2	1:2	10	10
P.K.....	M	75	0.01	0	0	0	0
M.T.....	F	50	0.001	0	1:8	0	100,000
M.R.....	F	36	0.001	0	0	0	1,000
F.J.....	F	58	0.001	0	0	10	1,000
M.F.....	F	21	0.001	0	0	0	100
P.G.....	F	26	0.001	0	0	10	100
E.M.....	M	60	0.001	0	0	0	0
M.C.....	F	66	0.001	0	0	0	0

TABLE I (continued)

Subject	Sex	Age	Amount injected	Agglutinins (serum dilution)		Mouse protection (fatal doses per 0.2 cc.)	
				Be-fore	After	Before	After
		years	mgm.				
C. Intracutaneous							
J.O'C.....	M	75	0.15	0	1:128	0	1,000,000
N.R.....	F	78	0.1	0	1:4	0	100,000
J.W.....	F	66	0.1	1:2	1:64	1,000	1,000,000
C.D.....	M	13	0.1	0	1:4	0	1,000
J.O'L.....	M	57	0.1	0	0	10,000	10,000
L.A.....	F	49	0.1	0	0	0	0
J.C.....	M	63	0.01	0	1:128	10	1,000,000
S.H.....	F	62	0.01	0	1:64	0	100,000
J.C.....	F	72	0.01	0	1:8	0	1,000,000
A.S.....	F	54	0.01	0	1:4	0	1,000,000
H.B.....	F	23	0.01	1:8	1:16	10,000	10,000,000
M.H.....	F	50	0.01	0	1:2	0	10,000
M.M.....	F	40	0.01	0	1:2	0	100,000
A.G.....	F	38	0.01	0	0	0	10
M.W.....	F	50	0.01	0	0	0	0
T.J.....	M	63	0.001	1:2	1:4	10	10,000
B.H.S.....	M	18	0.001	0	0	10	1,000
R.S.....	M	45	0.001	0	0	100	1,000
C.D.....	F	76	0.001	0	0	0	10
P.S.....	M	34	0.001	0	0	0	0
D.C.....	M	54	0.001	1:2	0	0	0
E.H.....	M	45	0.0001	0	1:8	0	10,000
A.I.....	M	32	0.0001	0	0	0	10,000
J.T.P.....	M	62	0.0001	0	1:2	0	1,000
B.A.....	M	57	0.0001	0	0	100	100
T.C.....	M	66	0.0001	0	0	0	0
D.N.....	M	44	0.0001	0	0	0	0
E.D.....	M	68	0.0001	0	0	10	0

amounts of the carbohydrate were used there was a steady decline both in the percentage of subjects showing a response and in the amount of antibody stimulated in each subject. 3. The amount used being the same, the carbohydrate was most effective when injected intracutaneously and least when given intravenously. This was particularly true with doses of 0.1 mgm. or less. 4. The optimum response was obtained with 1.0 mgm. given subcutaneously. A similar response followed intracutaneous injections of 0.15 to 0.01 mgm. 5. The 5.0 mgm. doses failed to stimulate antibodies in most of the subjects to whom they were given intravenously, whereas 1.0 mgm. administered by the same route yielded some response in every subject. 6. Doses of 0.001 mgm. or less gave only low grade and inconsistent responses. 7. As in previous studies with other types of pneumococci (3, 4), protective antibodies were demonstrated more readily than were agglutinins.

As regards the effectiveness of the Type VIII soluble antigen given by various routes, the results of the present study in human subjects are not in accord with those which have been obtained in

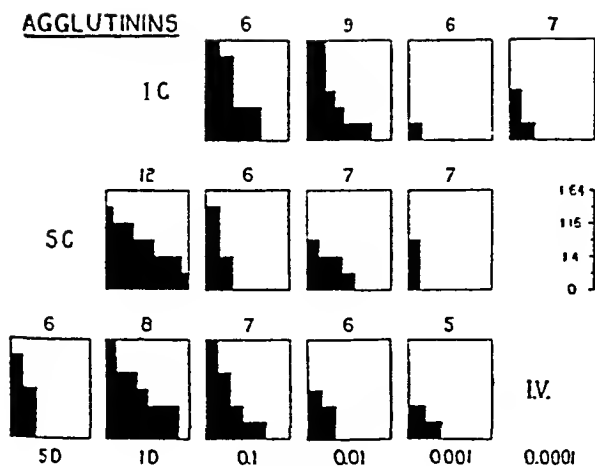


FIGURE 1

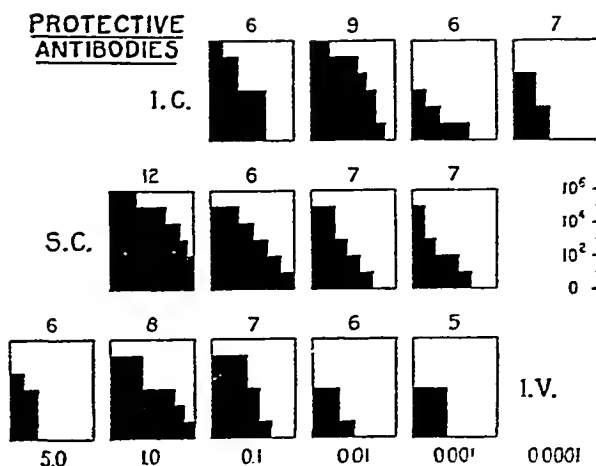


FIGURE 2

## EXPLANATION OF FIGURES 1 AND 2

Each block represents the homologous type-specific antibody response of a group of patients receiving the same dose of Type VIII specific carbohydrate by the same route. The number of individuals in each group is indicated above the block. The blocks from left to right represent groups of subjects injected by the same route, viz., I.V. = intravenous, S.C. = subcutaneous and I.C. = intracutaneous. The blocks from above down represent groups which received the same dose of the carbohydrate; the amount, in milligrams, is indicated at the bottom of the figures.

The solid portions represent the increase in antibodies following the injections. The height of the solid portions represents the titer of antibody, and the width of each portion represents the percentage of subjects acquiring that titer. The scale on the right represents the maximum titer, if none was present before the injection, or the maximum increase in titer.

Figure 1 represents the agglutinin response; the scale represents serum dilutions.

Figure 2 shows the mouse protective antibody response; the scale represents fatal doses protected by 0.2 cc. of serum.

animals with the whole pneumococcus cell. Stillman (5) obtained a progressively smaller type-specific antibody response in rabbits to Type I pneumococci given by the intravenous, intraperitoneal, intramuscular, and subcutaneous routes, respectively. Furthermore, Julianelle (6) was unable to demonstrate an appreciable titer of type-specific antibodies in the sera of rabbits inoculated with Type I or Type III pneumococci intracutaneously.

Of particular interest is the failure to obtain a response, in most instances, when 5.0 mgm. were given intravenously; whereas the 1.0 mgm. injections by the same route were almost constantly effective. Such a phenomenon was noted by Schiemann and Casper (7), who found that small doses of specific precipitable substance of the pneumococcus protected mice against subsequent fatal injections of live organisms, whereas 100 times the same dose failed to protect.

In our investigation, serum was obtained for the study of antibodies in most of the subjects at

weekly intervals for three or more weeks after the injection of carbohydrate. At the end of the first week only slight amounts of antibody could be demonstrated in the sera of a few individuals. This was true, in general, for each of the different amounts of material given by the various routes although some of the subjects who received intravenous injections did show relatively high titers after one week. As a rule, the maximum titers were obtained two weeks after injection. In some subjects receiving subcutaneous or intracutaneous doses, however, the titer continued to rise during the third week. In a small group of subjects whose sera were studied at intervals of two days, the increase in the titer of antibodies occurred between the seventh and ninth days, and the rise continued at a slower rate during the next five days. Similar results were obtained, in the latter experiments, both with intravenous and with subcutaneous injections.

In addition to tests for the homologous type of antibodies, all of the sera were examined for Type

III and either Type I or Type II agglutinins and, in most instances, tests for mouse protective antibodies against these types of pneumococci were carried out. Heterologous type-specific agglutinins were not demonstrated. In occasional subjects, heterologous type-specific protective antibodies were found to appear or to increase in titer in the sera obtained after the injection of Type VIII specific carbohydrate (Table II). The titers of such antibodies were always low and, with one exception, they were found to be active against the related Type III pneumococcus. In one individual, a 100-fold increase in Type I protective antibodies occurred in addition to the appearance of the Type III antibodies. Appreciable increases in phagocytic titer, without the development of demonstrable protective antibodies, were demonstrated for Type I pneumococci in two subjects and for Type II in two other individuals.

TABLE II

*Development of mouse protective antibodies against heterologous types in subjects receiving injections of the specific carbohydrate of the Type VIII pneumococcus*

Subject	Type VIII injection		Heterologous protective titer*		
	Route	Dose mgm.	Type	Be- fore	After
M.A.....	Intravenous	5.0	III	10	100
J.B.....	"	5.0	III	10	100
C.McF.....	"	1.0	III	10	100
D.B.....	"	1.0	III	0	10
G.M.....	"	0.1	III	100	100,000
F.S.....	Subcutaneous	1.0	III	0	100
W.H.M.....	"	1.0	III	10	100
J.C.....	Intracutaneous	0.01	III	0	100
			I	10	1,000
H.B.....	"	0.01	III	100	1,000
A.S.....	"	0.01	III	0	10
A.G.....	"	0.01	III	0	10

\* Fatal doses neutralized per 0.2 cc. serum.

For the homologous type-specific antibody response see preceding table.

#### SUMMARY

The specific carbohydrate of the Type VIII pneumococcus stimulates the production of ho-

mologous type-specific antibodies when given to human subjects by the intracutaneous, subcutaneous or intravenous route. The optimum dose was found to be 1.0 mgm. given subcutaneously. Smaller doses were more effective when administered intracutaneously than by the other routes. Doses of 1.0 or 0.1 mgm. were more effective when given subcutaneously than when injected intravenously. An intravenous dose of 5 mgm. failed to stimulate antibodies in most individuals, whereas 1.0 mgm. given in this manner caused a response quite constantly. A small amount of antibodies against Type III pneumococci were demonstrated in the sera of occasional subjects who had received injections of the Type VIII carbohydrate.

The authors are grateful to Dr. Rachel Brown for the supply of Type VIII carbohydrate used in this study. To her and to Dr. Augustus B. Wadsworth they are indebted for their generous cooperation and continued interest.

#### BIBLIOGRAPHY

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## TOGETHER WITH A CONSIDERATION OF THE RELATIONSHIP OF FETAL HEMATOPOIESIS TO MACROCYTIC ANEMIA OF PREGNANCY AND ANEMIA IN INFANTS<sup>1,2</sup>

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(From the Department of Medicine and the Surgical Hunterian Laboratory, the Johns Hopkins University, Baltimore)

(Received for publication July 3, 1935)

It is well known that the red corpuscles of the newborn are larger than those of the normal adult. Little study has been made, however, of the size and number of the corpuscles of the fetus. The purpose of this communication is to describe certain observations on the size and number of the red cells in the blood of the fetus and newborn of several species of mammal; namely, man, rabbit, pig, rat, cat and dog. In the species examined, it has been found that low erythrocyte counts and large red corpuscles are consistently found in the fetus, and that the younger the fetus the lower is the red cell count and the larger the red corpuscles. As compared with the blood of the adult of the same species, there is in the fetus what may for descriptive purposes be spoken of as "anemia" of the macrocytic type. As the fetus develops, the erythrocyte count rises and the mean size of the red corpuscles diminishes in a manner which reminds one of the changes which take place in the blood of patients with pernicious anemia during the response to liver therapy.

### REVIEW OF LITERATURE

Malassez (1) in 1875 and Cohnstein and Zuntz (2) in 1884 observed that the erythrocyte count in the early stages of development of the fetus (rabbit, dog, sheep) is low, and that it gradually increases. In 1889, the former measured the diameters of the red corpuscles (3) of a human fetus of four and one-half months and found the cells to be larger than those of adult blood. The exceptionally large size of the earliest nucleated red corpuscles of the embryos of mammals was noted much earlier than 1889, however, for Milne-Edwards (1857) (4) quotes Prévost, Wagner, Gulliver and Bischoff in this connection.

Jolly (5, 6) studied the blood of rat fetuses and newborn, and pointed out that in this species the red cell count even at birth is only about one-fourth of that of the adult whereas in the rabbit and human newborn the erythrocyte count is essentially the same as in the adult. Nicholas and Bosworth (7) observed an increase of hemoglobin from 30 to 65 per cent in rat fetuses ranging from the twelfth day of the fetal period to the newborn stage. Kindred and Corey (8) found increasing erythrocyte counts in rat fetuses 15.4 to 43.0 mm. in length (sixteenth to twenty-second day). Smith (9) reported diameter measurements as well as red cell counts in rats from two days before birth until the adult stage was reached. She found a gradual increase in the number of corpuscles until the twenty-third day of life when their number began to increase rapidly, attaining the adult values at about three months of age. Throughout this whole period the mean diameter of the red cells, their color index and variability, and the proportion of reticulocytes, gradually decreased.

Knoll (10, 11) has recorded successively increasing red cell counts in human fetuses one to six months of age.

Zeidberg (12) studied rabbit fetuses and found an increase in hemoglobin from 8 to 12 grams during the last third of pregnancy (twenty-second to thirty-second day) and an increase in packed red corpuscles from 28 to 42 per cent. He noted, moreover, that the proportion of basophilic red corpuscles decreased during this period from 28 per cent to 4 per cent. von Deseö (13) seems to have been the first to measure the mean volume and hemoglobin content of the red corpuscles in fetal blood. In 25 beef fetuses ranging from 2½ to 9 months of age he observed, as the age of the fetuses advanced, an increase in the erythrocyte count from 3.74 to 7.80 million, in hemoglobin from 7.65 to 10.83 grams and in volume of packed red corpuscles from 34.0 to 42.3 cc., whereas the mean corpuscular volume decreased from 90.9 to 54.2 cμ., mean corpuscular hemoglobin fell from 20.5 to 14.0 micrograms and mean corpuscular hemoglobin concentration fluctuated between 20.9 and 25.6 per cent.

### MATERIAL AND METHODS

Through the courtesy of the Department of Obstetrics, blood was obtained from 12 obviously non-viable human fetuses removed by hysterectomy. Blood was secured by cardiac puncture immediately following removal of the

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fetus. The approximate age of the fetuses was estimated from the crown-rump length and the entire length on the basis of the charts of Keibel and Mall (14) and Streeter (15). Blood was also obtained, by jugular puncture, from 3 premature infants at the Harriet Lane Home. The values for the blood of newborn infants which are used for Figure 4 represent the average of 31 determinations made immediately after birth, and 18 determinations made at intervals until the twenty-fifth day of life (16).

*Rabbits* of blue-black Dutch stock and of mixed laboratory stocks were mated in our own laboratory and, since the buck and doe were together for three or four hours only, the age of the rabbit fetuses studied could be estimated with accuracy. The fetuses were removed by hysterectomy and the blood immediately obtained. Whenever possible, blood was secured by cardiac puncture. In the case of very small fetuses the heart was cut open and the blood was drawn into a capillary pipet as it welled out. In order to obtain sufficient amounts of blood, in a number of instances the blood of several fetuses from the same uterus was mixed.

The blood of *rat* fetuses was obtained in the same way as that of the rabbit fetuses. The rats were not deliber-

ately mated, however, and the age of the fetuses had therefore to be calculated from weight and crown-rump length on the basis of data published by Donaldson (17). The age of newborn rats was determined from accurate litter records. The rats were of an inbred laboratory stock on a standard diet (18).

*Pig* fetuses were obtained in a neighboring abbatoir. The uteruses of pregnant sows were brought to the laboratory immediately after the animals had been slaughtered and the fetal blood was there collected without delay. In no instances had clotting commenced, and in some of the pig fetuses the heart was still beating when the blood was withdrawn. Blood was frequently collected from the umbilical cord, it having been found that the erythrocytic content of blood obtained in this way was the same as that of blood taken from the heart. In the case of the smaller pig fetuses, blood was taken directly from the heart in the manner already described. The age of the pig fetuses was estimated from their weight and crown-rump length on the basis of Warwick's data (19). Blood of newborn pigs was obtained by cardiac puncture on animals at the U. S. Research Center, Beltsville, Maryland, through the courtesy of Dr. Hugh McPhee.

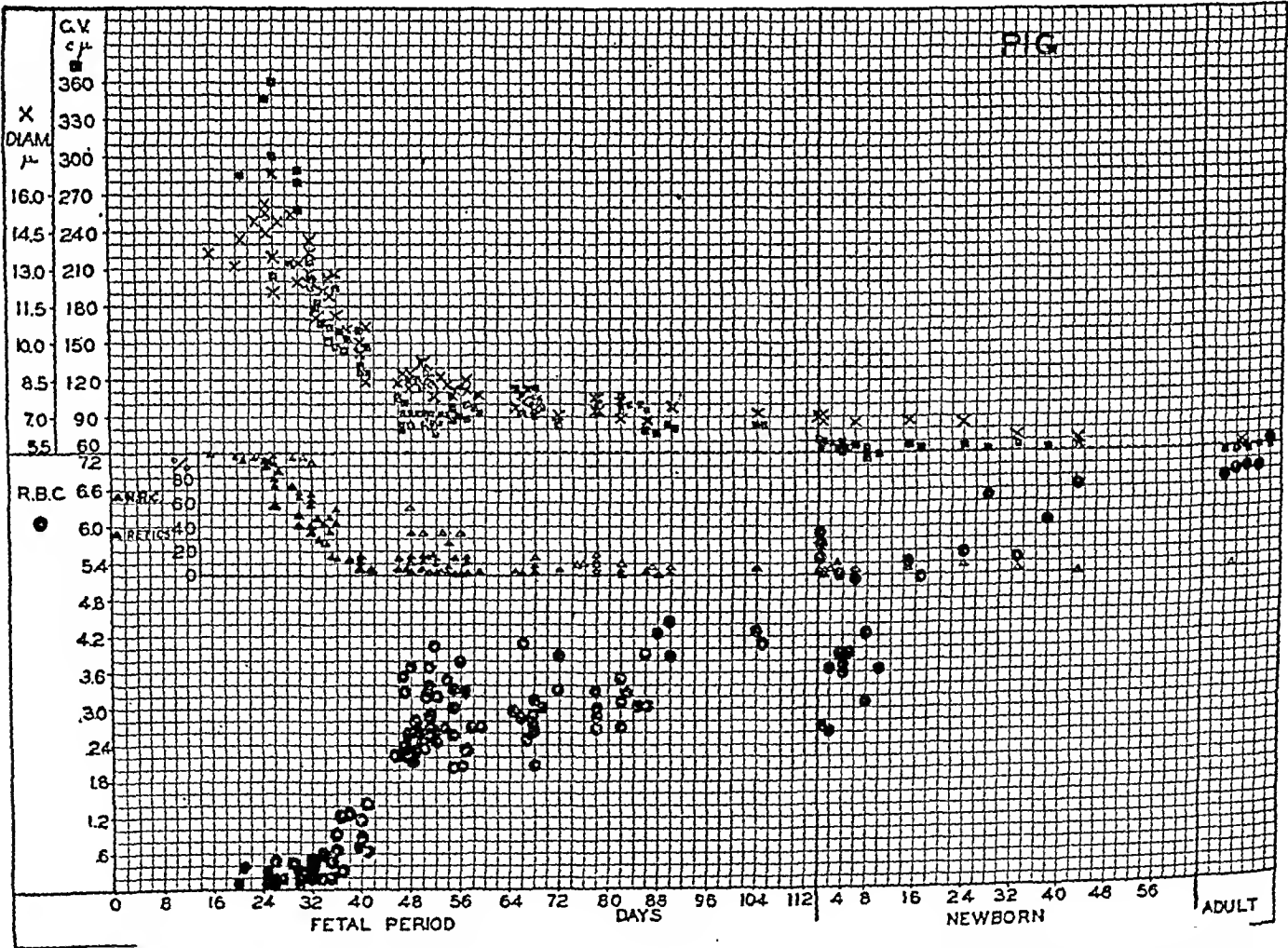


FIG. 1. ERYTHROCYTE COUNTS (○, MILLIONS PER C.M.M.), MEAN CORPUSCULAR VOLUME (■, CUBIC MICRONS), MEAN DIAMETER IN WET PREPARATIONS (X, MICRONS), PROPORTION OF NUCLEATED RED CORPUSCLES (▲) AND OF RETICULOCYTES (Δ) IN THE BLOOD OF 98 PIG FETUSES, 22 NEWBORN PIGS, AND 5 ADULT PIGS.

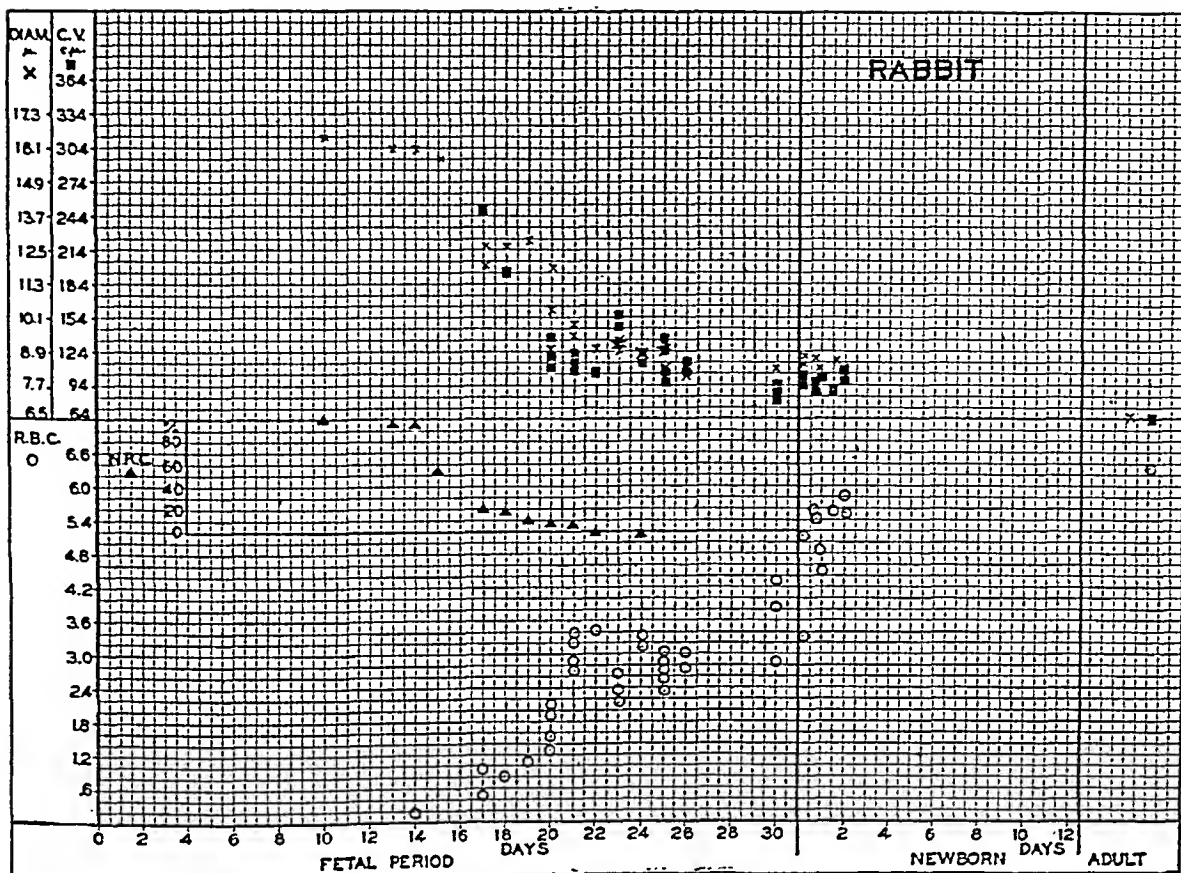


FIG. 2. ERYTHROCYTE COUNTS (○, MILLIONS PER C.M.M.), MEAN CORPUSCULAR VOLUME (■, CUBIC MICRONS), MEAN DIAMETER IN WET PREPARATIONS (×, MICRONS) AND PROPORTION OF NUCLEATED RED CORPUSCLES (▲) IN THE BLOOD OF 34 RABBIT FETUSES AND 8 NEWBORN RABBITS, COMPARED WITH AVERAGE VALUES FOR THE ADULT RABBIT.

Blood of newborn dogs and cats was obtained by cardiac puncture on animals born in the laboratory. The age of fetuses was estimated from weight and crown-rump length (20, 21).

The blood was collected either in heparin or without an anticoagulant, it having been found that coagulation is extremely slow and imperfect in the blood of young fetuses. Hemolyzed specimens were discarded.

Fresh blood preparations and blood smears were made, and two diameters of 25 to 100 unselected red corpuscles in the wet and in the dried, stained films (Wright's stain) were measured by means of a calibrated ocular micrometer. The proportion of nucleated corpuscles was determined in the stained preparations. Reticulocyte counts were made in wet preparations to which a small quantity of brilliant cresyl blue (1 per cent in normal saline) had been added. Erythrocyte counts (two in each instance), hemoglobin and hematocrit determinations, and calculations of the mean volume and hemoglobin content of the red corpuscles were made as described elsewhere (22, 23).

Two chief sources of error must be kept in mind in in-

terpreting the results of these studies: (1) except in the case of the rabbit fetuses, the age of fetuses has been estimated from data for length and weight and is therefore only approximate; (2) when the fetuses were very small, such minute amounts of blood were available that values in the smaller fetuses for hemoglobin, volume of packed red cells, and mean volume and hemoglobin content of the red corpuscles cannot be considered as representing the same degree of accuracy as is possible when studying the blood of adults (23). Erythrocyte counts, however, may be considered as being quite accurate in almost all instances, and diameter measurements represent of course the same degree of accuracy for fetuses of all sizes.

#### OBSERVATIONS

Details of the blood studies are published elsewhere (24). Erythrocyte counts, mean corpuscular volume, mean diameter (in wet preparations) and the proportion of nucleated red corpuscles and



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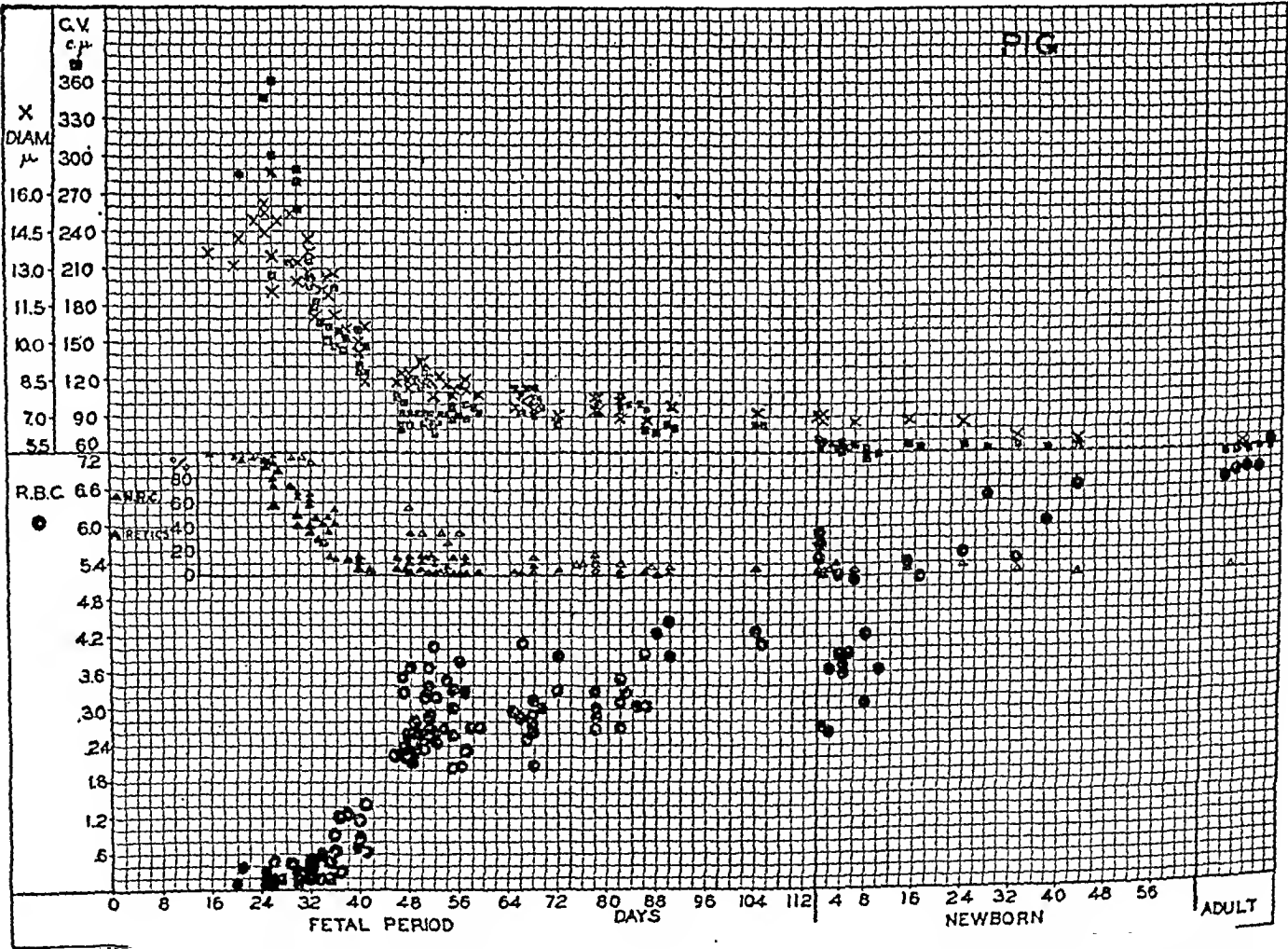


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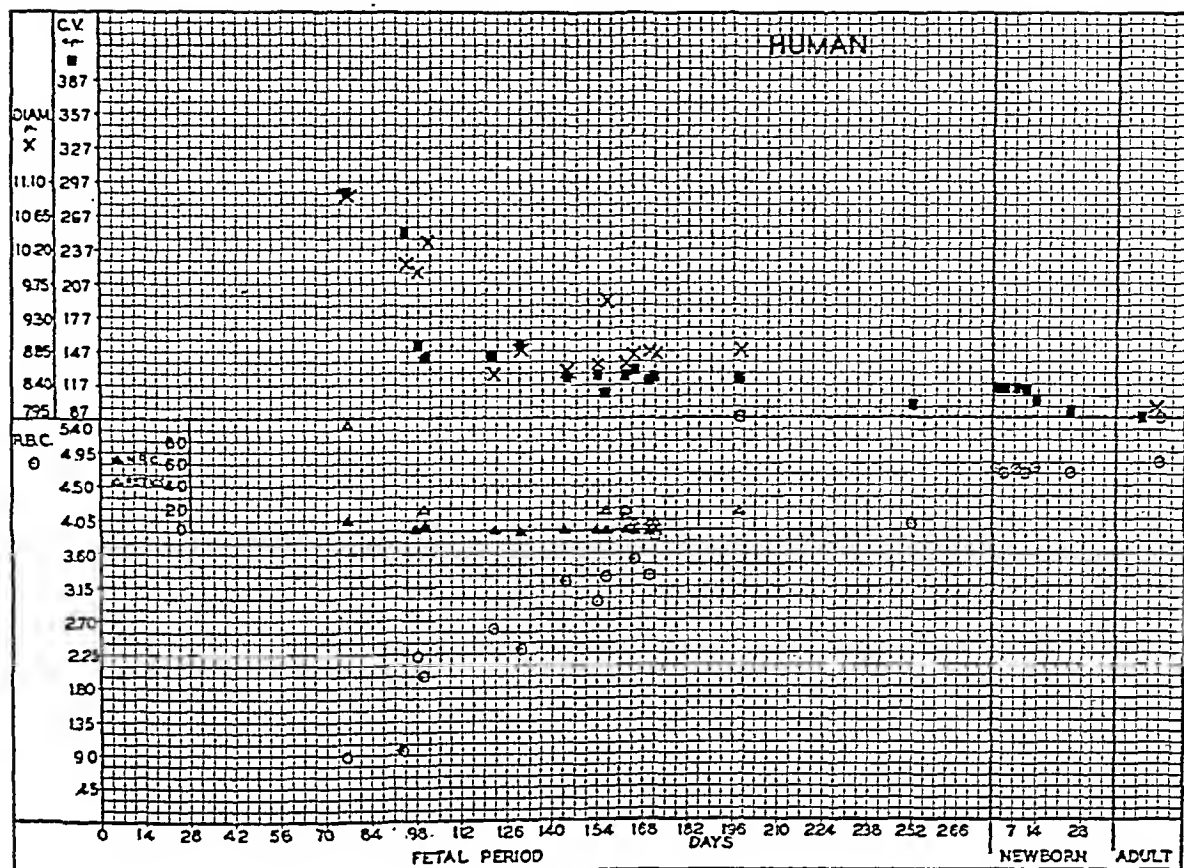


FIG. 4. ERYTHROCYTE COUNTS (○, MILLIONS PER C.M.M.), MEAN CORPUSCULAR VOLUME (■, CUBIC MICRONS), MEAN DIAMETER IN WET PREPARATIONS (X, MICRONS), PROPORTION OF NUCLEATED RED CORPUSCLES (▲) AND OF RETICULOCYTES (△) IN THE BLOOD OF 12 HUMAN FETUSES AND 3 PREMATURE INFANTS, COMPARED WITH AVERAGE VALUES FOR 49 FULL TERM NEWBORN INFANTS AND THE NORMAL ADULT.

similar to those which take place during the normal development of the blood corpuscles in the fetus. The senior author has described elsewhere (25) the variations which take place in mean corpuscular volume in cases of pernicious anemia during liver treatment. There is a striking resemblance between the curves which represent the changes in pernicious anemia and those shown in Figures 1 to 4.

Superficial examination suggests that the macrocytosis observed in the blood of the fetus is much greater than that of pernicious anemia. Mean corpuscular volumes as great as even five and six times the normal have been found in the blood of very young fetuses, whereas in pernicious anemia it is unusual to find an increase in mean corpuscular volume to as much as even twice normal. It must be borne in mind, however, that in

pernicious anemia erythrocyte counts below 1 million cells are quite unusual and values lower than 0.5 million are rarely, if ever, encountered. The extremely high values for mean corpuscular volume were observed only in those instances in which the erythrocyte counts were as low as 5 per cent of those of the adult, and less; that is, they correspond in terms of human blood to red cell counts lower than 250,000 per c.mm. When corpuscular volumes corresponding to similar reductions below the normal adult red cell count are compared (see Figures 5, 6 and 7) the degree of macrocytosis in the fetus is found to be quite similar to that of pernicious anemia.

Not only is the mean size of the red corpuscles in fetal blood greater than that of the red cells of the adult, but a great degree of anisocytosis is present. As the fetus develops, this anisocytosis

reticulocytes in the blood of fetuses and newborn of the pig, rabbit, rat and man are shown in Figures 1 to 4. The data are so plotted that a comparison with the values found in the adult may be readily made. It will be noted that the erythrocyte count is very low in young fetuses and increases as the fetus develops. The mean volume and mean diameter of the red corpuscles are at first very much greater than in the adult, and decrease as the red cell count rises.

In a few instances marked differences in the erythrocyte counts of litter mates, even greater than one million cells, were encountered. Usually, however, the counts fell within a range of 300,000 cells per c.mm. It is of interest that the mean sizes of the red corpuscles of litter mates agreed without exception within limits of technical varia-

tion even when great differences in red cell counts occurred.

Even at birth the erythrocyte count was found to be lower than that of the adult, and the corpuscles were larger than is normal for the adult. This was found to be especially true of the rat, as Jolly had noted (5), but was observed also in the blood of the other species of mammals examined. Our studies in the rat indicate that almost two months elapse before the values of the adult are attained.

#### *Comparison of the blood of the fetus with macrocytic anemia in the human adult*

In many respects the changes which have been observed in the blood of patients with pernicious anemia under the influence of liver therapy, are

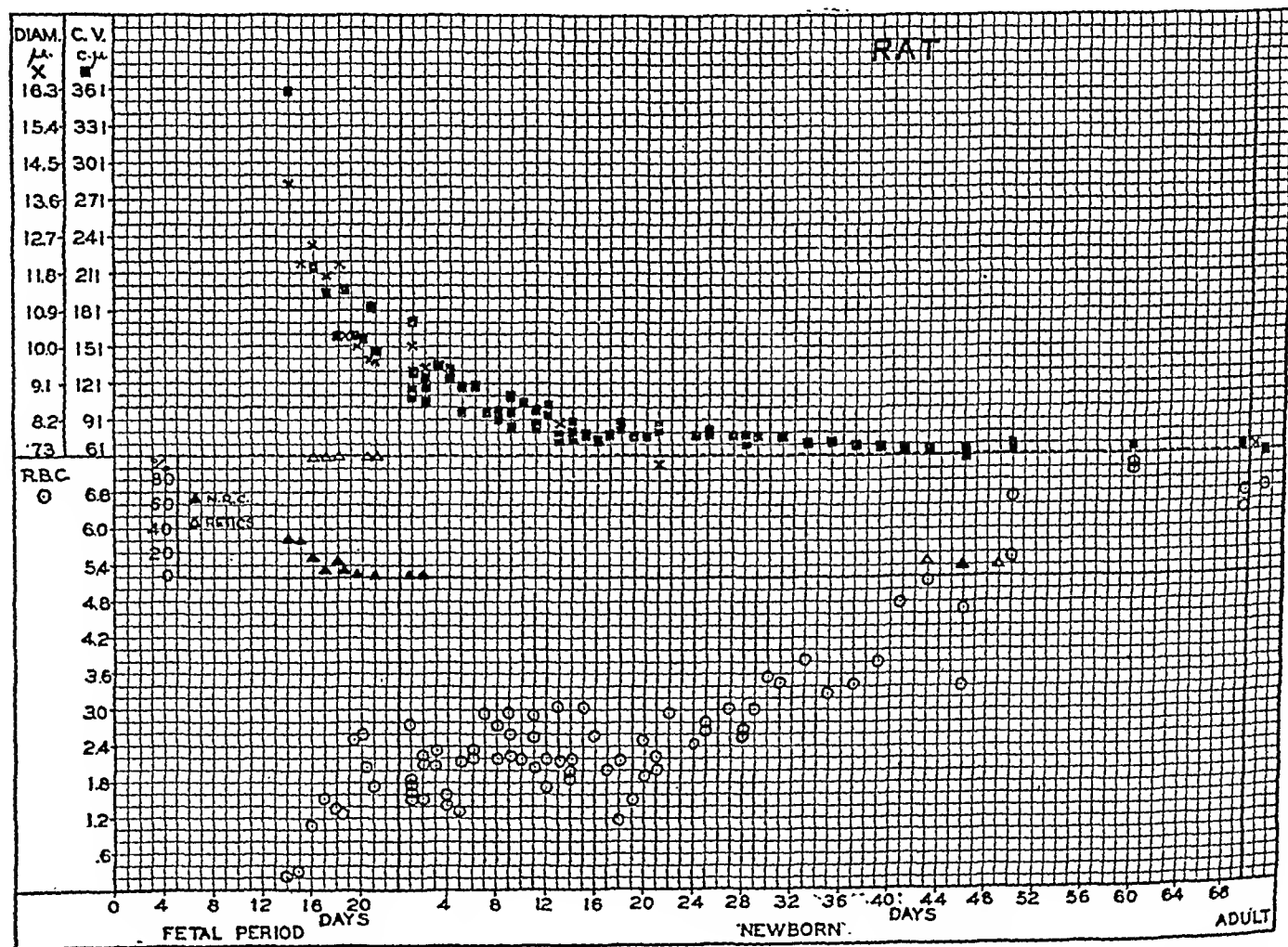


FIG. 3. ERYTHROCYTE COUNTS (○, MILLIONS PER C.MM.), MEAN CORPUSCULAR VOLUME (■, CUBIC MICRONS), MEAN DIAMETER IN WET PREPARATIONS (X, MICRONS), PROPORTION OF NUCLEATED RED CORPUSCLES (▲) AND OF RETICULOCYTES (Δ) IN THE BLOOD OF 11 RAT FETUSES AND 67 NEWBORN RATS, COMPARED WITH AVERAGE VALUES FOR THE ADULT RAT.

diminishes in a manner which again brings to mind the changes which occur in pernicious anemia under the influence of liver therapy (Figures 8 and 9).

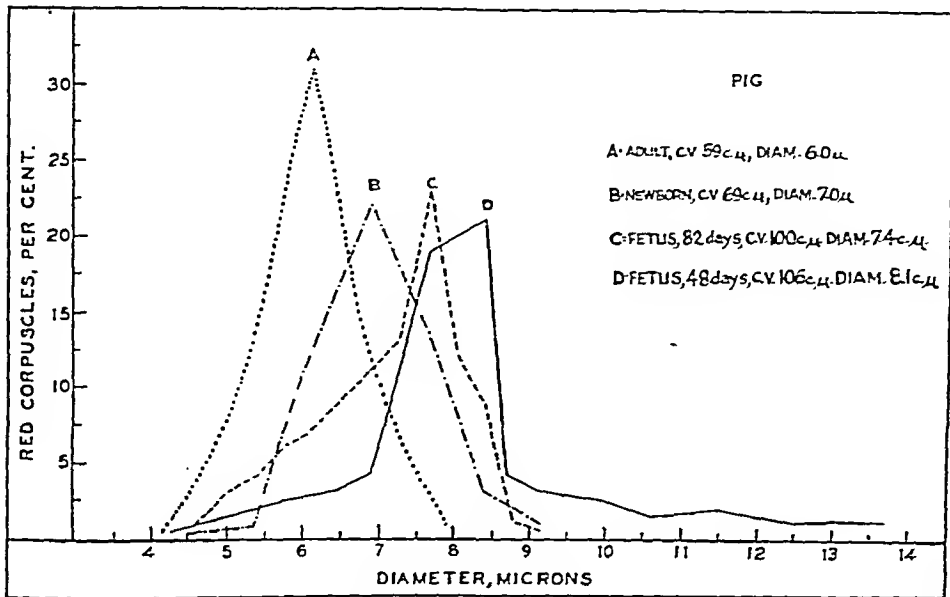


FIG. 8. DISTRIBUTION OF THE DIAMETERS OF THE RED CORPUSCLES IN THE BLOOD OF PIG FETUSES COMPARED WITH THOSE OF THE BLOOD OF THE NEWBORN AND THE ADULT PIG.

Cells measured in fresh (wet) preparations.

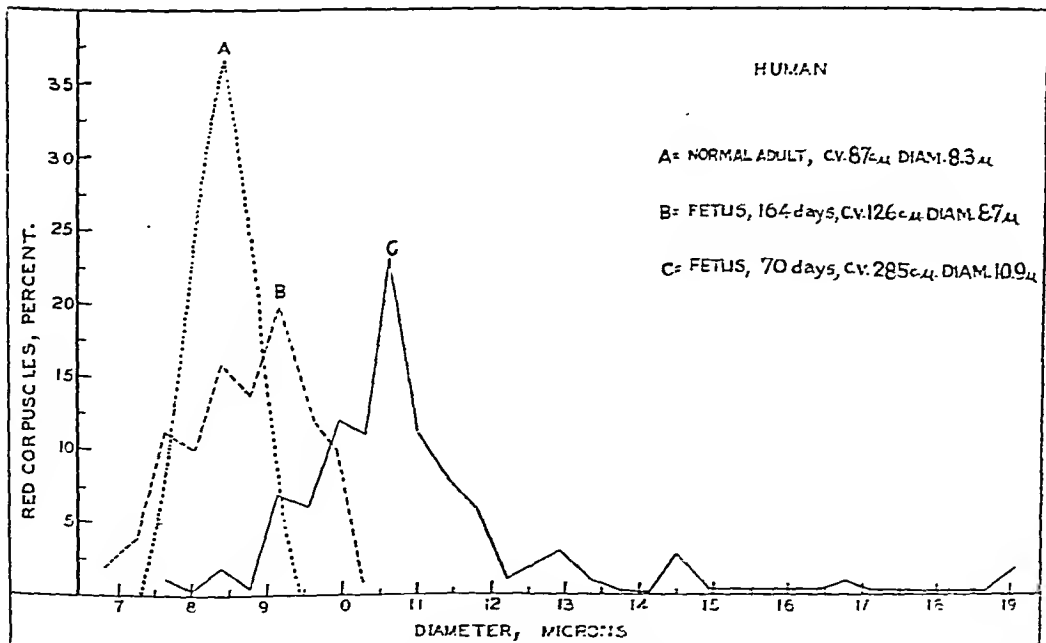


FIG. 9. DISTRIBUTION OF THE DIAMETERS OF THE RED CORPUSCLES IN THE BLOOD OF HUMAN FETUSES COMPARED WITH THE ERYTHROCYTES OF THE NORMAL ADULT.

Cells measured in fresh (wet) preparations.

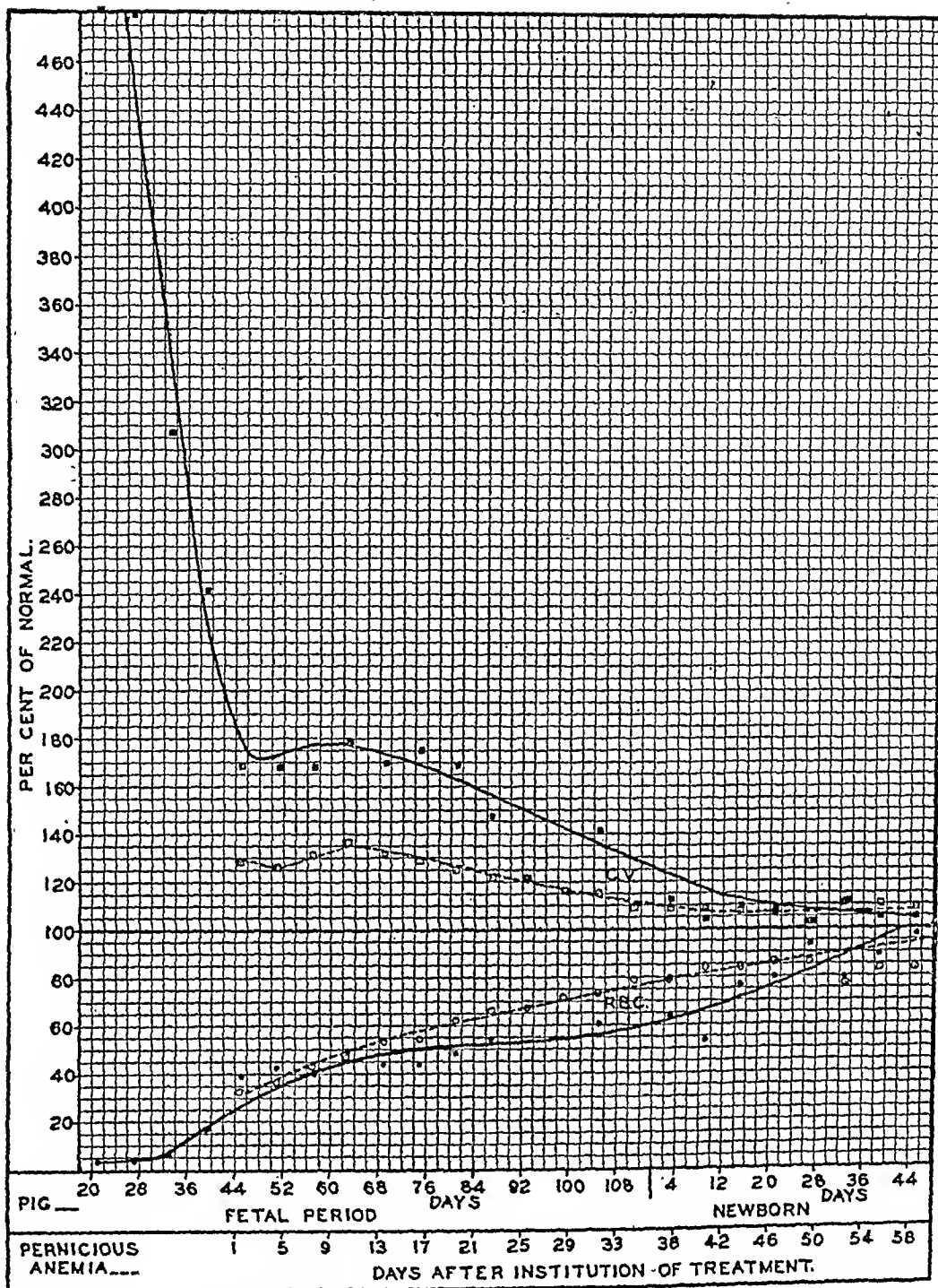


FIG. 7. SMOOTH CURVES FOR COMPARISON OF THE ERYTHROCYTE COUNT AND MEAN CORPUSCULAR VOLUME IN 98 PIG FETUSES AND 22 NEWBORN PIGS WITH THOSE OF 9 CASES OF PERNICIOUS ANEMIA EXAMINED DAILY DURING THE RESPONSE TO LIVER THERAPY (245 DETERMINATIONS).

The observations in the pigs were grouped according to age in periods of 6 days each and the average for each group calculated. The observations in the cases of pernicious anemia were grouped according to time following the institution of treatment in periods of three days each and the average for each of these groups calculated. The results are plotted in proportion to the normal for the adult of each species (pig, 6.93 million red corpuscles, 59 c. $\mu$ . mean corpuscular volume; human, 5.1 million red corpuscles, 87 c. $\mu$ . mean corpuscular volume). These averages are shown in the chart as black circles for pig erythrocyte counts and open circles for human erythrocyte counts; black squares for pig mean corpuscular volumes and open squares for human mean corpuscular volumes. The curves were drawn with the aid of a curve rule so that they would fit as well as possible the plotted averages. The complete line curves refer to the pig fetuses and newborn, and the interrupted line curves represent the blood changes in the cases of pernicious anemia.

A number of objections to such a comparison come to mind; thus, the calculated reticulocyte counts represent the maximum expected number of reticulocytes in a series of cases of pernicious anemia, and do not indicate the changes which would occur in the blood of a single individual. Again, the formulae for expected reticulocyte response were derived empirically from observations in cases of pernicious anemia in which the reduction in the red cell count was never as low as in some of the instances for which the formulae are here employed. It is evident that the data shown in Table I afford only a very rough comparison; yet it is one which is useful in giving some conception of the intensity of blood formation in the fetus.

Certain points of difference between the blood in cases of pernicious anemia and that of the fetus must be mentioned. Probably the most striking difference is that poikilocytes, although present in fetal blood, are fewer in number, and the extremely bizarre forms so characteristic of Addisonian anemia, are rarely, if ever, found (Figure 10). Again, it is not unusual to find in pernicious anemia a few erythrocytes which are poorly filled with hemoglobin. In the fetal bloods studied such cells were not observed. Finally, as judged by icterus index, blood destruction was no greater in the fetuses of the pig, rabbit, rat, dog and cat than in the adult of each of these species. In the human fetuses, however, the icterus index was usually high, being 25 in two instances, 20 in one, 17 in two and 16, 15, 12, and 7, respectively, in four other fetuses. The Van den Bergh test, carried out in only two of these bloods, gave a strong indirect reaction in one instance in which the icterus index was 25 and a slight indirect reaction in the instance in which the icterus index was 15.

#### DISCUSSION

It is fully appreciated that the morphologic similarity between the blood in cases of pernicious anemia and that of the developing fetus may be but a superficial one, and the agent which influences one set of changes may be quite different from that which leads to the other. Yet the resemblance is so great that comparison seems justified. There is, moreover, similarity in other respects. In the bone marrow of patients dying of

pernicious anemia during relapse and in marrow obtained by biopsy at this time, extensive proliferation of primitive cells is found (28, 29). The megaloblast of Naegeli is described by him as occurring only in the blood of the early embryo and in that of pernicious anemia (30). Even extramedullary blood formation occurs in pernicious anemia as was first noted by Meyer and Heineke (31) and by Schridde (32). It was, in fact, the finding of foci of extramedullary hematopoiesis in the spleens of some cases of liver disease in which we were interested, and the observation that such foci were associated in most instances with macrocytic anemia (33), that led us to undertake the studies here recorded.

It is our impression, derived from the study of the changes which take place in the size and number of the red corpuscles of the fetus, as well as in the proportion of erythroblasts and reticulocytes, that in many respects the *blood of the developing fetus resembles that of cases of pernicious anemia which are being subjected to an effective, continuous and extremely potent stimulus to blood formation.*

It would also appear from our studies that the macrocytosis of the newborn, frequently considered to be the result of physicochemical changes in the blood, really represents a final stage in the development of the blood in the fetus. In comparison with the blood in pernicious anemia, the blood of the newborn is similar to that of cases in incomplete remission. An apparent contradiction to this hypothesis is the polycythemia which has been reported in the blood of newborn infants by some investigators (34). Actually, greatly varying results have been recorded (35), and it is doubtful if in the newborn infant which has not become dehydrated, the red cell count is even as high as in the normal adult. In studies made with Eastman and Jack (16) one of us found erythrocyte counts in newborn infants which were slightly lower than the average values for adults. Guest's recent observations on the blood of the human newborn (36) are in exact agreement with our own.

Castle and his associates (37) have demonstrated that pernicious anemia develops as the result of the deficiency of an "antianemic principle" which is formed by the interaction of an



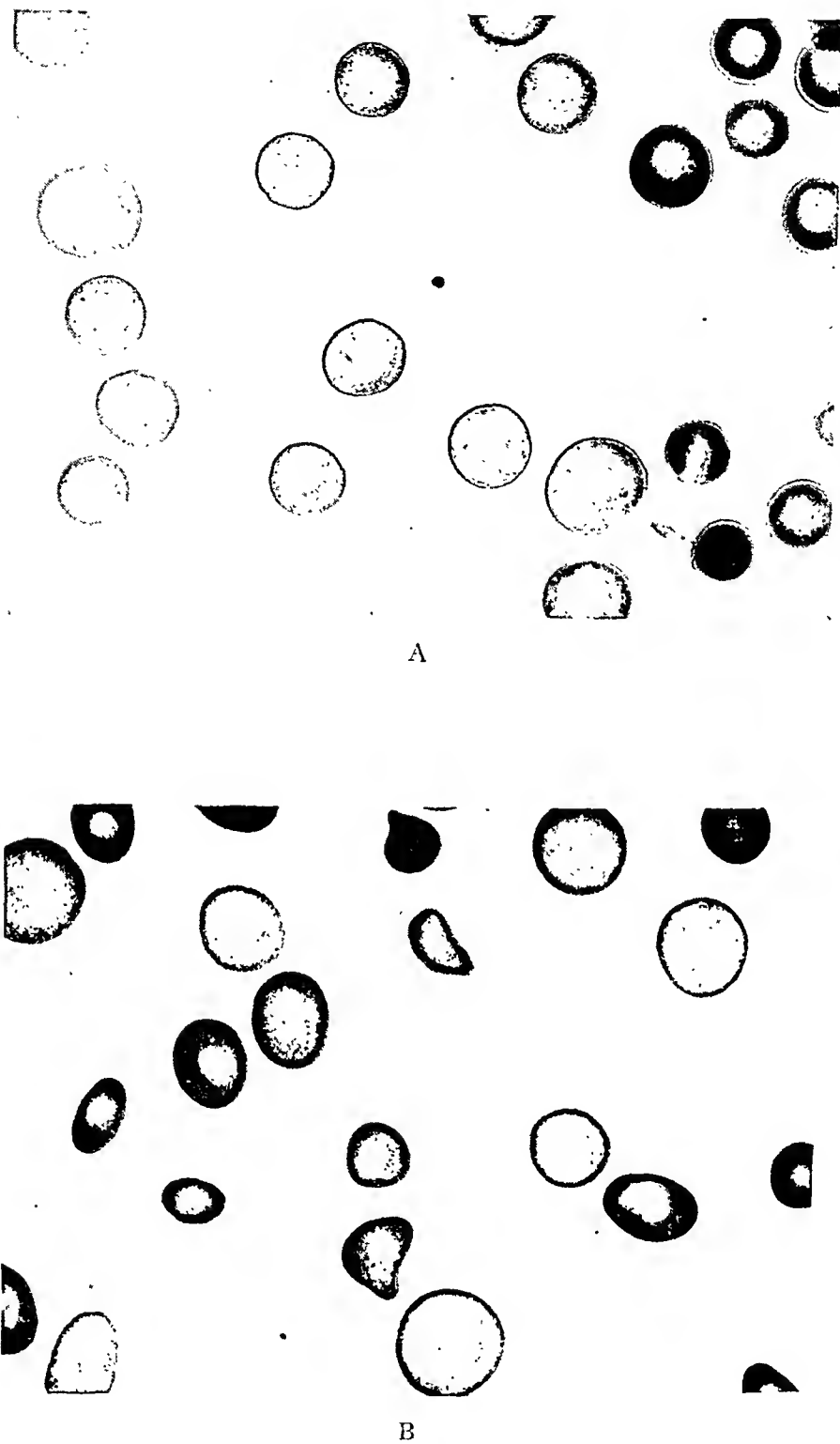


FIG. 10. THE BLOOD OF A HUMAN FETUS COMPARED WITH THAT OF A CASE OF PERNICIOUS ANEMIA. Magnification 1170.

A. Human fetus of approximately 98 days. R.B.C. 2.2 million, mean diameter (stained preparation)  $8.45 \mu$ .

B. Pernicious anemia. R.B.C. 1.68 million, mean diameter (stained preparation)  $8.21 \mu$ .

"extrinsic factor" in the diet and an "intrinsic factor" secreted by the stomach. It is now well known that the administration of this principle, which is found in greatest concentration in liver, is followed by active blood formation, progressively decreasing macrocytosis and a rapid disappearance of anemia. *It seems quite plausible that the antianemic principle of Castle may be the same or very similar to the substance which causes the blood of the fetus to develop in the manner described.*

Presumably, there is no gastric digestion in the fetus and no combination of "intrinsic" and "extrinsic" factors takes place as occurs after birth. If a substance similar to Castle's antianemic principle influences the blood of the fetus, it probably comes through the placental circulation from the stores of the mother. It is of interest to point out that antianemic principle has been found not only in the liver, kidney and brain, but also in the placenta (38).

Investigations are in progress which seek to determine whether or not there is any relationship between fetal hematopoiesis and that observed in cases of pernicious anemia treated with liver. The clinical implications of such a relationship, if it can be proved to exist, are of great importance. It is now generally agreed that in Addisonian anemia the degree of anemia is closely dependent on the degree of deficiency of antianemic principle. If there is a similar relationship between the quantity of antianemic principle available and the formation of blood in the fetus, *the effect of a deficiency of this substance on the mother and on the infant, may be readily visualized.*

If the fetus derives antianemic principle from the mother, this may be expected in some instances to cause so great a depletion in the stores of the mother that she herself develops macrocytic anemia. It is characteristic of the so-called "pernicious anemia" of pregnancy that it develops only during pregnancy, it is relieved by liver therapy, it does not recur following delivery even though liver therapy is discontinued, and it may reappear at a succeeding pregnancy (39). These characteristics conform exactly to what might be expected if the fetus withdraws antianemic principle from the mother. That per-

nicious anemia of pregnancy is uncommon, indicates that the majority of pregnant women form during the normal processes of digestion, quantities of antianemic principle which are adequate to meet their own requirements and those of the fetus. Strauss (39) has observed in cases of macrocytic anemia in pregnancy that the quantity of gastric secretion is often deficient and, in addition, the diet taken by the mother is frequently lacking in animal protein. In these instances the formation of antianemic principle falls below the needs of mother and fetus and macrocytic anemia develops.

An infant for whom an inadequate amount of antianemic principle has been made available during the gestation period; may be expected to develop anemia characterized by macrocytosis and by ready response to the administration of liver extract. Such an anemia might be expected to be more common in premature infants, for these have been afforded a shorter period for the acquisition of antianemic principle, and in twins if they have developed, during the intrauterine period, under conditions in which only a limited amount of antianemic principle is available. If the lack of antianemic principle in the infant is the result of deficient formation of the material by the mother, it would not be unexpected that the resulting anemia would reappear in the progeny of succeeding pregnancies.

That these considerations are not hypothetical is suggested by the experience of pediatricians. Parsons (40) refers to a "primary anemia of the newborn" which is macrocytic in type and "frequently occurs in premature children or in twins and particularly in premature twins." The erythroblastosis of the newborn which has recently received a great deal of attention (41, 42) is characterized by familial history, macrocytic anemia, erythroblastosis, increased blood destruction, increased deposition of iron pigment in the tissues and extensive extramedullary foci of blood formation. These findings at once bring pernicious anemia to mind. Liver therapy does not seem to have received serious trial in these cases but it is interesting to note, even though it appears to be an isolated instance, that Bernheim-Karrer and Grob (43) reported successful prophylaxis by the daily feeding of 100 grams of liver during the



last ten weeks of pregnancy, in a mother who had previously given birth to two infants who died of "icterus gravis." In other types of anemia in infants, liver therapy has been reported as being of value (44, 45).

Clinical observations quite analogous to those postulated as associated with deficiency of anti-anemic principle, have been reported in connection with iron-deficiency anemia in pregnant women and their offspring. Hypochromic microcytic anemia is not uncommon in pregnancy, and is generally considered to be the result of the combined effects of deficient diet, faulty gastric secretion, and the increased requirements for iron due to the needs of the fetus (39). Infants born of mothers with this type of anemia, are not anemic at birth, but they frequently develop moderate to severe degrees of anemia during the first year of life (46). This anemia may be relieved by administering iron to the infants, and it may be prevented by giving iron to the mothers during pregnancy (46).

There is little information concerning the blood of infants born of mothers suffering from either true pernicious anemia or macrocytic anemia of pregnancy. It is generally stated that such infants are not anemic at birth. Even if this is true, it does not necessarily invalidate the hypothesis presented for, in the case of iron deficiency at least, the fetus seems to withdraw iron for blood-building at the expense of the mother and in spite of her increasing anemia.

#### SUMMARY

1. Determinations of the erythrocyte count, mean corpuscular volume, mean diameter and proportion of nucleated red corpuscles and reticulocytes in the blood of fetuses and newborn of man, the pig, rabbit and rat, are graphically recorded.

2. It is shown that in very young fetuses of the species examined, the erythrocyte counts are very low and the red corpuscles are very large when compared with the values for red cell count and size in the normal adult of each species. As the fetus develops the erythrocyte count rises, and the red corpuscles become smaller. The proportion of nucleated red corpuscles decreases very

rapidly, while the percentage of reticulocytes diminishes more gradually.

3. In all of the species examined, some macrocytosis was found to be still present at birth; and in the rat, rabbit, pig, cat and dog the erythrocyte count was lower than that of the adult. In man, the erythrocyte count is approximately normal at birth. In the newborn dogs examined, the counts were substantially below the adult values and in the newborn rats they were approximately one-third of those of the mature rat.

These observations suggest that the macrocytosis of the newborn represents a final stage in the normal development of the blood.

4. Comparison is made between the development of the blood in the fetus and the changes which occur in the blood of cases of pernicious anemia in response to liver therapy.

5. The blood of the fetus resembles in many respects that of cases of pernicious anemia which are being subjected to an effective, continuous and extremely potent stimulus to blood formation.

6. It is suggested that the antianemic principle of Castle may be responsible for the described changes in the blood of the fetus, and that this principle passes to the fetus from the stores of the mother. On this hypothesis it is possible to visualize the mode of development of "pernicious anemia" of pregnancy. The significance of this conception in regard to anemia in infants is also considered.

We are indebted to Dr. I. P. Earle and to Mr. J. H. Zeller of the U. S. Research Center, Beltsville, Maryland, for securing for us blood from newborn pigs; and to Messrs. William J. Schmidt and William A. Oktavec, Jr., as well as Miss Mary Smith, for technical assistance. Mr. Milton Kougel made the microphotographs.

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# A CLINICAL AND EXPERIMENTAL STUDY OF THE STABILITY OF COLLOID OSMOTIC PRESSURE OF SERUM PROTEIN

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The work of earlier investigators, Govaerts (1), Baráth (2), Cope (3), Peters et al. (4) and Kylin (5) has given the impression that the colloid osmotic pressure is not only lowered with decrease in serum protein but its specific pressure (pressure per unit of protein) is also diminished. The lowering of specific pressure has usually been related to a greater decrease in the albumin fraction of the proteins than that of the globulin, so that the A:G ratio becomes reduced or inverted. However, in all of these reports, the pressure readings were taken at the end of twenty-four hours or longer, and probably do not represent the highest point on the pressure curve. Wells et al. (6) have recently discussed the validity of determining the colloid osmotic pressure by a rapid method devised by them. They also presented data on hypoproteinemic sera which gave low specific pressures, and referred to the fact that the pressure in many cases after rising to some extent began to fall within a few hours. They attempt to explain this by putrefactive change or molecular aggregation of serum protein (6, 7). In the method reported here the possibility of contamination of the serum with bacteria has been excluded. Measurement of the colloid osmotic pressure of the serum proteins of nephrotic patients has revealed a decrease in the stability of the pressure readings in those instances where the concentration of proteins approached the edema level. In most of the cases the maximum pressure was obtained in three to five hours, and thereafter began to fall gradually. This was in marked contrast to the prolonged plateau of nearly constant pressure observed when the concentration of protein was normal.

While good correlation was observed between

the maximum pressure and the content of total protein, there was little evidence of correlation between the ratio of albumin: globulin and the maximum pressure. The data show that the pressure per gram of protein may actually be somewhat greater when the serum proteins are low than when their concentration falls within the normal range.

## METHOD

Blood was taken with the least possible stasis from the antecubital vein, while the patient was recumbent in bed. Precautions were observed to insure sterility of the serum during collection of the blood and during the subsequent centrifugation and separation of the serum from the clot.

The specific gravity of the serum was determined by Hammerschlag's method (8), the refractive index by Abbe's refractometer at constant room temperature of 23° C. The total content of protein, albumin and globulin were determined by Howe's method using 1 cc. of serum for each sample (9). The determination of colloid osmotic pressure was carried out with the apparatus devised by Fellows (10), similar in principle to that of Verney (11). The osmometer has the advantage that the osmotic pressure can be followed at any desired interval of time. Some modifications were made on the instrument itself, the stopcock being removed from its original position to the upper end of the graduated portion of the capillary tube, and the hole on the side of the osmometer tube being omitted in order to minimize the danger of any leakage of serum.

As the dialyzing agent a modified Ringer's solution consisting of 0.8 per cent sodium chloride and 0.042 per cent potassium chloride was used. The osmometer was inclosed in a large glass tube for sterilization, and autoclaved for 15 to 20

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TABLE II

*Colloid osmotic pressure of reduced stability in patients with low serum protein*  
(Through the courtesy of Dr. S. W. Clausen 3 pediatric cases are included in this group.)

Name	Age and sex	Specific gravity	Total protein	A : G ratio	Colloid osmotic pressure				Stability	Remarks
					Highest pressure	Specific pressure	Lowest pressure	Specific pressure		
	years		per cent		mm. H <sub>2</sub> O	mm. H <sub>2</sub> O	mm. H <sub>2</sub> O	mm. H <sub>2</sub> O	hours	
J.S...	68 ♂	1.030	5.20	2.17	275.5	53.0	253.7	48.7	8	Hypertension, cardiac insufficiency
A.B...	68 ♀	1.030	5.14	1.74	252.3	49.2	230.3	44.9	8	Arteriosclerosis, cardiac insufficiency
E.B...	12 ♀	1.027	5.30	1.02	240.1	45.4	210.0	39.6	7	Chronic hemorrhagic nephritis
G.S...	45 ♂	1.026	5.05	0.63	221.5	43.9	195.8	38.8	6	Lymphatic leukemia, edema
V.N...	2 ♂	1.026	4.50	0.88	192.0	42.7	138.0	30.7	6	Nephrosis
J.M...	8 ♂	1.027	4.50	0.79	184.5	41.0	130.2	29.0	5	Nephrosis
J.H...	23 ♀	1.025	4.47	2.02	240.0	53.7	180.0	40.3	5	Chronic nephritis
E.W...	50 ♀	1.025	3.52	0.91	209.5	59.4	122.0	34.7	4	Carcinomatosis
Average						48.5		38.3		

Similar observations have been made by Weech, Snelling and Goettsch (12). The difference between the values for specific gravity given in their paper and the values obtained here may be due to the fact that in the former plasma was used and in the latter serum. Moreover, different methods were used.

Our results on the colloid osmotic pressure of serums having concentrations of protein within normal limits correspond on the whole to those reported by Baráth (2), Oelkers (13), Bonsmann (14), Tada and Nakazawa (15) and Kylin (5). Drinker and Field (17) have reported pressures for dog serum agreeing very closely with ours, especially in regard to the specific osmotic pressure of protein in normal dogs' serum. Identical types of pressure curves were obtained for human and dog sera during the time of equilibration (see Figures 1A and B).

Inspection of Figure 1 shows that the pressure usually reaches a maximum within five or six hours, in eight hours at the most, and that it is maintained at this level over a period of twenty-four hours. These curves may be regarded as showing normal stability of colloid osmotic pressure of serum protein.

#### *Changes of stability of pressure in cases of low serum protein*

When the content of serum protein was lowered considerably the stability of the colloid osmotic pressure was found to be markedly reduced. Curves obtained from such sera are shown in Figure 2. The relation of reduced stability of pressure to lowered protein content was confirmed in forty determinations, all of which gave the same types of curves varying one from another only in degree of declivity. The pressure began to fall gradually as soon as its maximum height was attained, and the rate of falling seems to have been quite constant in its whole course. The height of the maximal point depended upon the total concentration of serum protein, and in cases of like protein content it was little affected by greater albumin: globulin ratios (Table III). The time at which the pressure began to fall and the rate of its fall seemed to depend on the total protein content but may also have had some relationship to the albumin: globulin ratio. In general, the higher the content of protein, the longer the period of time before the pressure began to decrease and the more slowly it fell. This relationship is shown in Figure 2B, in which three different cases have been compared.

Routine examinations for possible sources of error in the apparatus were made. Sterilization produced no change in the permeability of the membrane (DuPont number 300 plain transparent cellophane). The difference in time required for equilibration of various concentrations of diffusi-

## RESULTS

As may be noted, the specific gravity of the serum remains quite constant over a wide range of protein content, although there is some tendency for it to vary in the same direction as the concentration of protein (see also Table II).

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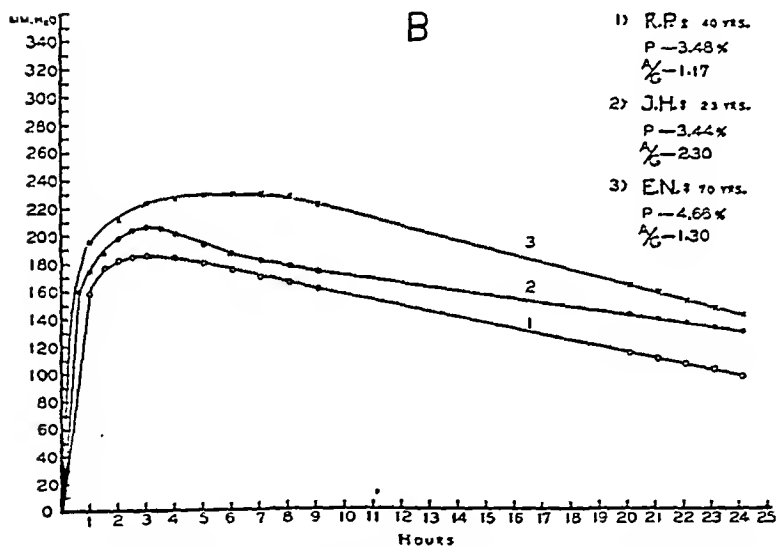
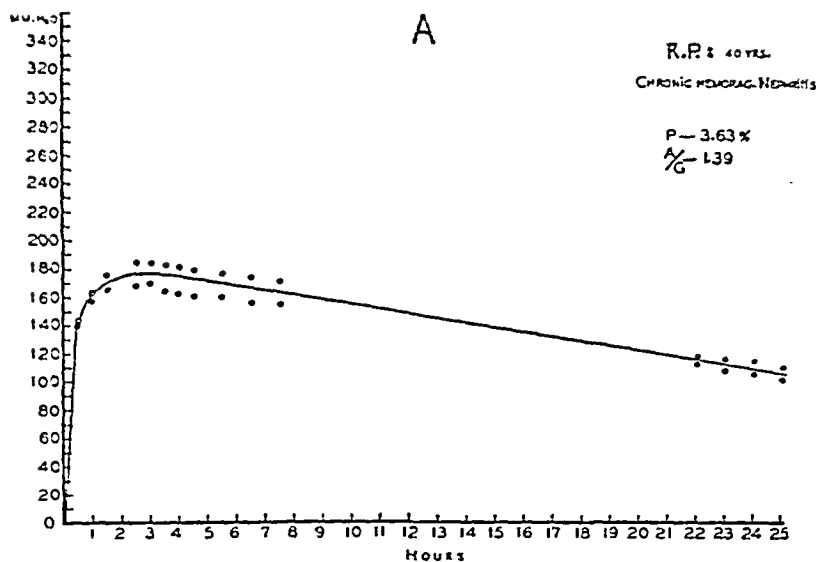


FIG. 2-A. THE CURVE OF REDUCED STABILITY OF COLLOID OSMOTIC PRESSURE OF SERUM PROTEIN IN PATIENT R. P.

P indicates the total protein content of serum, A/G indicates the albumin-globulin ratio, dots and circles represent duplicate analyses of the same serum.

FIG. 2-B. THE COMPARISON OF CURVES OF REDUCED STABILITY OF COLLOID OSMOTIC PRESSURE IN DIFFERENT PATIENTS.

Each point represents the average of duplicate determinations.

value for both the total and specific pressures. On the other hand, the average of two or three readings at short intervals of time at the highest part of the curve yields a value for total pressure approximating that obtained by multiplying the protein content by the average value of normal

specific pressure. For instance, the average of duplicate maximum pressures of serum proteins in Figure 2A were found to be 174.5 millimeters of water, the concentration of protein was 3.63 per cent, giving a specific pressure of 48 millimeters of water, which is close to our average



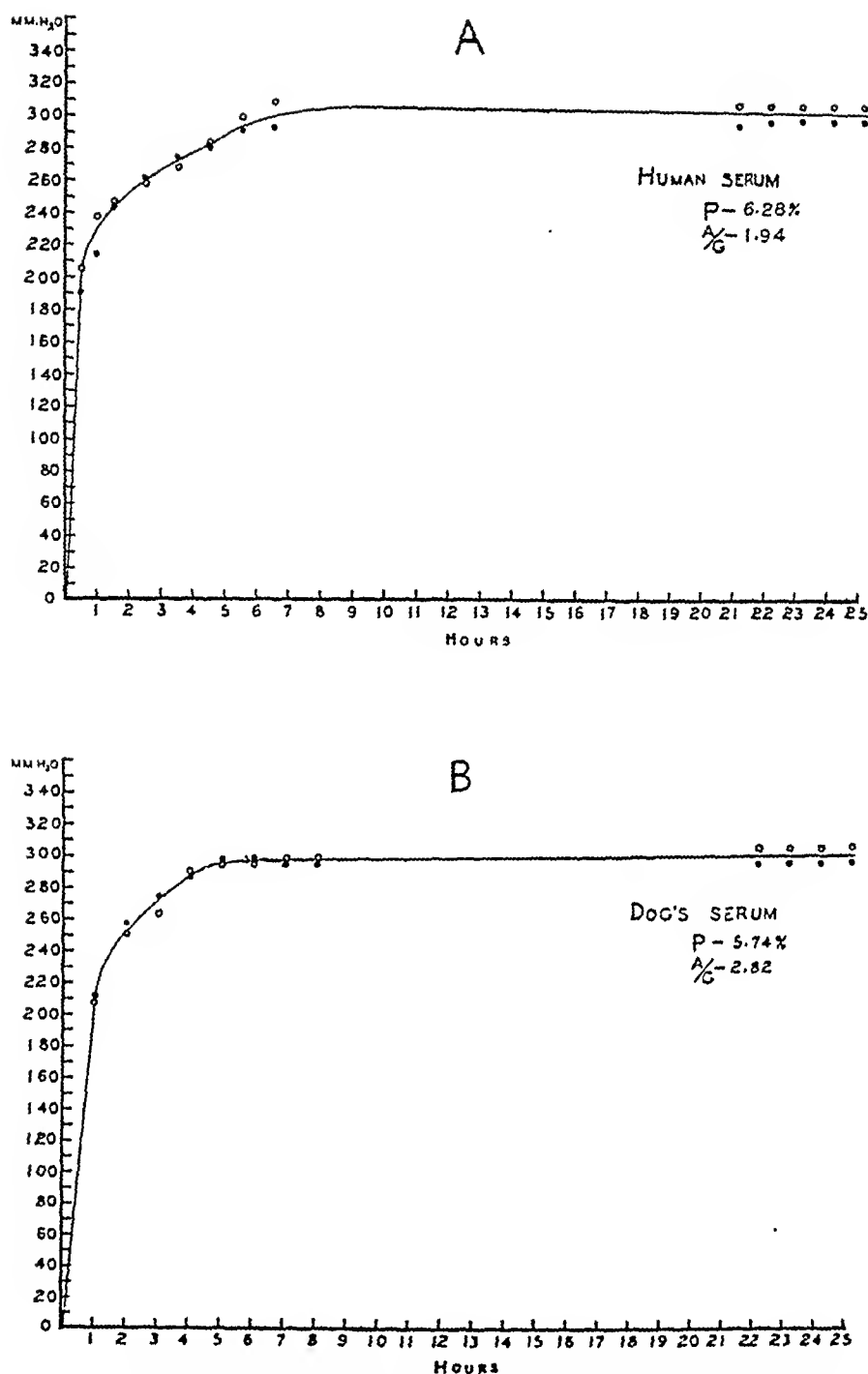


FIG. 1. THE CURVE OF NORMAL STABILITY OF COLLOID OSMOTIC PRESSURE OF SERUM PROTEIN IN HUMAN (A) AND IN DOG (B).

$P$  indicates the total protein content of serum,  $A/G$  indicates the albumin-globulin ratio, dots and circles represent duplicate analyses of the same serum.

Curves 1 and 2 are from sera having almost the same content of protein, and are roughly comparable. Curve 3 rose to the highest point, due to the higher content of protein and maintained its plateau of pressure longer than did Curves 1 and 2.

This raises the question as to what should be considered the real osmotic pressure of such pro-

teins. Practically, the readings of the manometers come so close to each other, even in the declining part of the curve, if read several times within an hour or two, that taking their average at the end of twenty-four hours may seem to be justifiable. Under such circumstances, the final reading of the pressure at the end of a period of twenty hours or longer gives a relatively low

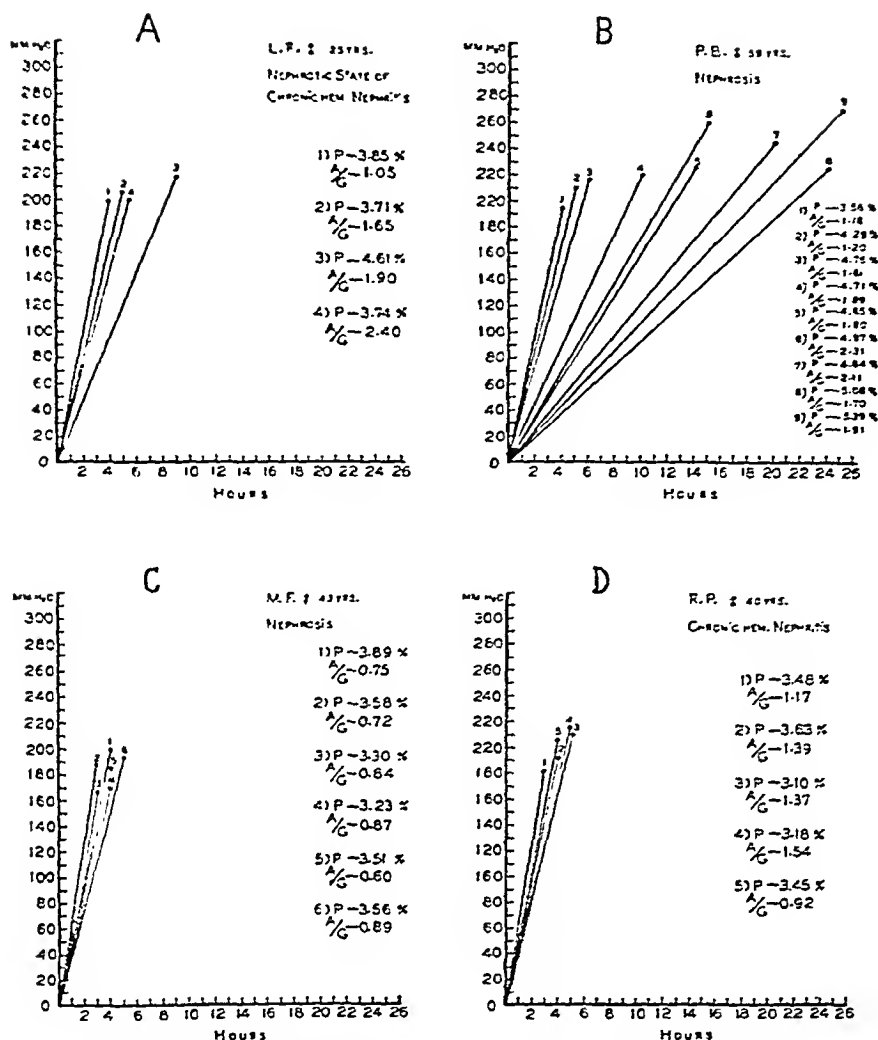


FIG. 3. THE CHANGE OF STABILITY OF COLLOID OSMOTIC PRESSURE OF SERUM DUE TO CHANGE IN CONCENTRATION OF PROTEIN.

Each point represents the average of duplicate determinations and gives the maximum pressure and the maximum duration of the stable period. P indicates the total protein content of serum, A/G indicates the albumin:globulin ratio.

*The effect of dilution of normal serum upon the stability of colloid osmotic pressure of serum protein*

Govaerts (1) assumed the cause of low osmotic pressure in nephrotic patients to be due to the dilution of the blood and the fall of the specific pressure of serum proteins. Verney (11) reported that artificial dilution of serum will cause a marked fall of specific pressure of serum protein. Kylin (5) recently found that the osmotic pressure of albumin or globulin per gram per 100 cc. of water differed according to the concentration of the solution used in the determination

of the pressure. When dilute solutions of these protein fractions were used, the specific pressure was far less than with higher concentration. Grönwall (16) also reported a similar relation between the specific pressure and the concentration of the serum protein.

Various dilutions of normal dog serum were prepared by the addition of 0.9 per cent saline at pH 6. All experimental manipulations were carried out under strictly aseptic conditions. After the osmotic pressure had been measured the protein content of the solution in the osmometer was determined by the refractometer. Although this

TABLE III  
Total and specific pressure of serum protein obtained at the highest part of pressure curve in markedly low protein cases

Name	Age and sex	Total protein	A : G ratio	Total pressure	Specific pressure	Remarks
		per cent		mm. H <sub>2</sub> O	mm. H <sub>2</sub> O	
L.R..	25 ♂	3.85	1.05	199.0	51.7	Nephrotic stage of chronic hemorrhagic nephritis
L.R..	25 ♂	3.74	2.40	201.2	53.7	Nephrotic stage of chronic hemorrhagic nephritis
L.R..	25 ♂	3.71	1.65	207.0	55.8	Nephrotic stage of chronic hemorrhagic nephritis
P.B...	59 ♂	3.56	1.18	192.3	54.0	Lipoid nephrosis
M.F..	43 ♀	3.58	0.72	188.0	52.5	Nephrosis
M.F..	43 ♀	3.56	0.89	197.2	55.3	Nephrosis
M.F..	43 ♀	3.23	0.87	171.0	53.0	Nephrosis
R.P..	40 ♂	3.48	1.17	182.9	52.5	Chronic hemorrhagic nephritis
R.P..	40 ♂	3.45	0.92	206.1	59.8	Chronic hemorrhagic nephritis
J.H...	23 ♀	3.44	2.30	198.3	57.7	Chronic nephritis
Average					54.6	

normal value of 49.9 millimeters. The average of the final readings were 110 millimeters of water as total pressure, giving a specific pressure of only 30.3 millimeters, which is comparable to the usual figure reported for hypoproteinemic sera by many workers.

Change of stability of colloid osmotic pressure with change of patient's condition

In three nephrotic patients and one with chronic hemorrhagic nephritis determinations of osmotic pressure were made at frequent intervals during the course of illness. Data are presented in Figure 3. In these charts the average pressure from duplicate determinations made at the peak of the curve has been plotted as the ordinate, and the time at which the pressure began to fall has been taken as the abscissa.

Figure 3*A* gives data from a case of chronic hemorrhagic nephritis in the nephrotic stage. Maximum pressures were proportional to the concentration of protein in the different samples of serum. As usual with hypoproteinemia, all pressure readings were labile.

Figure 3*B* presents data from a patient with

lipoid nephrosis with slight edema, who improved steadily while under observation. The osmotic pressure increased with the increase of protein in the serum. Stability also increased as the concentration of protein increased. The last examination (Line 9) showed almost a normal curve of stability with a pressure of 270 millimeters of water and a concentration of protein of 5.39 per cent, giving a specific pressure of 50.2 millimeters. Figure 3*C* presents a case of nephrosis with marked generalized edema. In six examinations of the serum the total proteins varied from 3.23 to 3.89 per cent with reversal of the albumin:globulin ratio. The maximum pressure varied with the protein content, and all pressure readings showed a marked and uniformly decreased stability. Figure 3*D* presents another case of chronic hemorrhagic nephritis with low serum proteins. The curves of the osmotic pressure closely resembled those shown in Figure 3*C*, the characteristic features being low A:G ratios and decreased stability.

Other data obtained from several patients with lower serum protein which have not been included in Table I or the figures will be found in Table II. As attention has already been directed to the fact that it is difficult to decide what may be the true osmotic pressure in such cases of reduced stability, two values are given, one obtained at the peak of the curve and the other at the end of twenty-four hours. The differences between the respective, total and specific pressures at these two intervals of time are frequently quite striking. Again, if one considers only the maximum pressure the specific pressure approaches the normal value. Low total proteins when combined with low A:G ratios seem to have been associated with the lowest pressure readings at the end of 24 hours.

Since the types of cases thus far considered include beside various nephropathies, cases of heart disease, lymphatic leukemia and carcinomatosis it seemed probable that hypoproteinemia itself rather than some specific constituent of the nephrotic serum was responsible for the lack of stability of the colloid osmotic pressure. Some experimental data in support of this hypothesis have been obtained.

method of estimating concentration of protein gives a somewhat higher value than the Kjeldahl method it was found sufficiently accurate for comparative purposes.

Since similar results were obtained whenever normal serum was diluted in the manner described, the discussion has been limited to a typical case. In Figure 4A, Curve 1 shows the behavior of the colloid osmotic pressure of the undiluted serum while Curves 2 and 3 show the pressures obtained after sufficient saline had been added to reduce the concentration of protein to 4.48 and 3.45 per cent respectively. The pressure of the diluted serum had a distinct tendency to fall after about seven hours, the sample with 3.45 per cent of protein falling more rapidly than the less dilute sample. The undiluted serum on the other hand maintained a constant pressure from the sixth to the twenty-fourth hour. The specific pressure of the normal serum was 54.5 millimeters of water, those of the diluted sera 47.5 and 43.5 millimeters respectively when computed at the end of twenty-four hours. However, the specific pressures, computed on the basis of maximum pressure, proved to be the same in all three samples.

Experiments were also performed, using sterile ultrafiltrate<sup>3</sup> from serum as the diluting agent. No difference was noted in the behavior of serum diluted with ultrafiltrate and that diluted with saline. The curves obtained were similar in all respects to those shown in Figure 4A.

Serum which had been diluted sufficiently to reduce its concentration of protein to about three or three and a half per cent frequently gave a higher specific pressure (computed from the maximum pressure) than did the undiluted serum. This tendency for lower concentration of protein to yield high specific pressures was found to hold also for abnormal sera having protein contents within the same general range (Table III).

*Change of stability of osmotic pressure produced by concentration of hypoproteinemic serum*

Serum from patients with hypoproteinemia was concentrated aseptically by ultrafiltration, and its colloid osmotic pressure compared with the original serum. Five experiments using samples from different patients gave similar results. The data

from a single experiment are presented in Figure 4B. Curve 1 represents the original serum containing 4.3 per cent of protein, Curve 2 that of the serum condensed to protein concentration of 5.4 per cent, and Curve 3 that condensed to a concentration of 6.22 per cent. It will be noted that the original serum had a relatively marked reduction in the stability of the osmotic pressure while the serum condensed to 5.4 per cent had improved in its stability. Further concentration to 6.22 per cent produced a curve of normal stability. When one compares the specific pressures of these three samples, using the maximum pressure as the point of reference they are found to be almost equal to each other, namely, 50.2, 51.7 and 50.6 millimeters of water. At the end of twenty-four hours Curves 2 and 1 representing the less concentrated sera gave specific pressures of 44.6 and 37.2 millimeters of water respectively. The behavior of the serum on concentration again suggests that the principal cause of reduced stability of colloid osmotic pressure is dilution of the serum with respect to protein.

#### DISCUSSION

Inability to maintain a constant level of pressure for more than a few hours seems to be a characteristic of hypoproteinemic sera, whether the lowering of the concentration of protein is produced by disease or dilution of normal serum. Although the present experiments fail to provide a basis for the interpretation of this phenomenon, they demonstrate that the maximum pressure developed by such sera is about the same as the pressure derived by multiplying the observed concentration of protein by the average specific pressure of normal serum.

The falling of the pressure which was regularly observed in hypoproteinemic sera seems to have been due to an unknown change in the protein occurring several hours after it had been placed in the osmometer. It is probable that a similar change does not occur in the body. It seems remarkable, however, that concentration of the protein was sufficient to abolish lability of pressure in such hypoproteinemic sera. Other factors, such

<sup>3</sup> Filtration was carried out with a Zsigmondy ultrafiltration apparatus (Pfaltz and Bauer Inc., New York) and an albumin proof ultrafine filter No. 1128. The filtrate obtained was water clear, of pH 8 and had a specific gravity of 1.014.

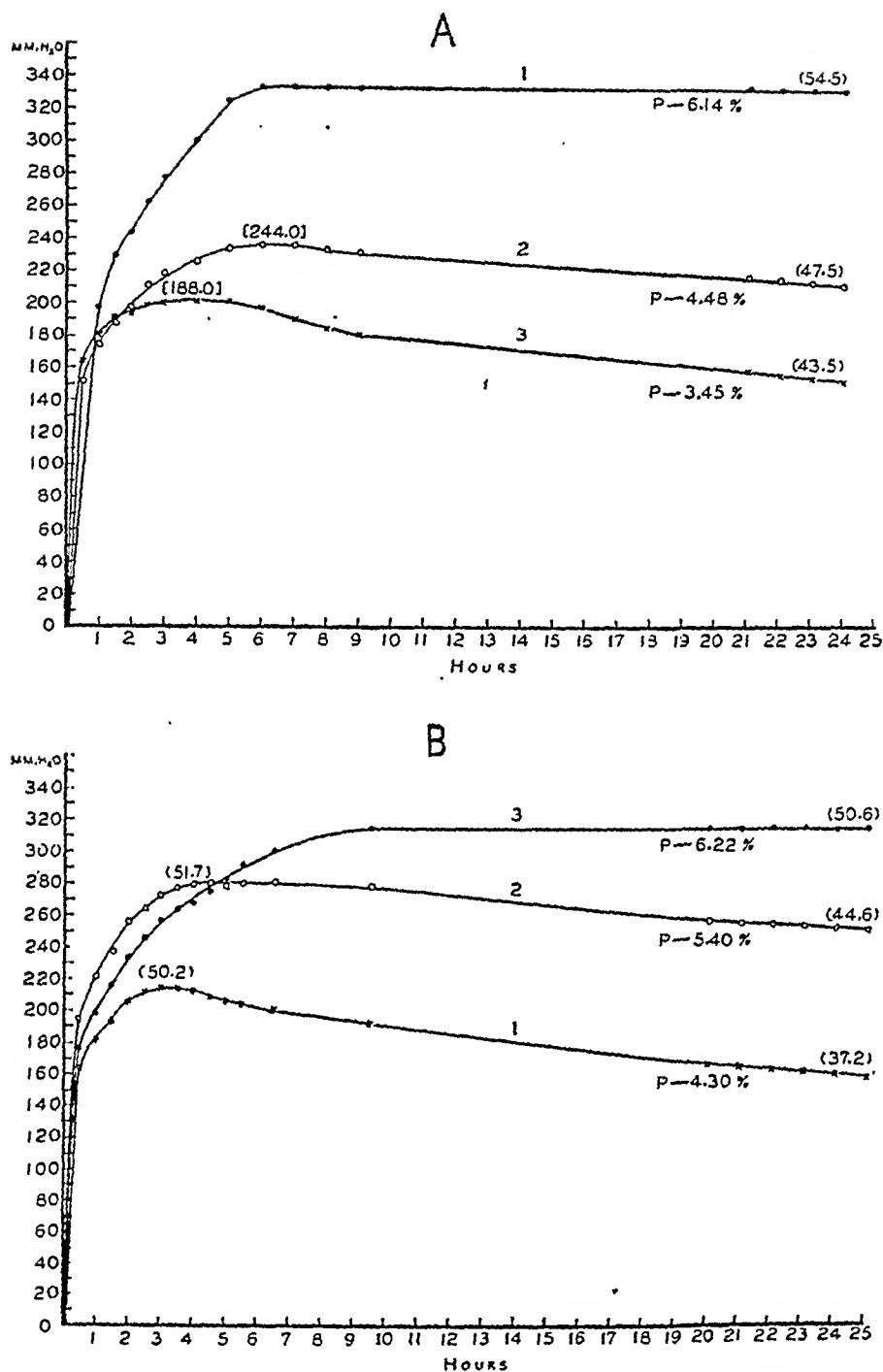


FIG. 4-A. THE EFFECT OF DILUTION ON THE STABILITY OF COLLOID OSMOTIC PRESSURE OF SERUM PROTEIN.

Curve 1 represents the pressure of normal serum from dog. Curves 2 and 3 represent those diluted with saline solution.  $P$  indicates the total protein content of serum, figures in parentheses give the specific pressure obtained at those points, figures in brackets give the predicted total pressure obtained from the protein content and the specific pressure in Curve 1.

FIG. 4-B. THE EFFECT OF CONCENTRATION ON THE STABILITY OF COLLOID OSMOTIC PRESSURE OF SERUM PROTEIN.

Curve 1 represents the serum from a patient, with pressure of reduced stability. Curves 2 and 3 represent those specimens condensed by ultrafiltration. Figures in parentheses give the specific pressures obtained at those points,  $P$  indicates the total protein content of serum.

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as alteration in pH of the serum during the period of equilibration, require further investigation in this connection.

It is evident that measurement of the colloid osmotic pressure at the end of twenty or twenty-four hours gives only an approximate idea of the total pressures which hypoproteinemic sera are capable of developing. In general, the lower the concentration of protein the less representative will be the twenty-four readings. A:G ratios appear to have had little or no effect upon the development of maximum pressures. On the other hand, ratios below one seem to have been more frequently associated with low pressures at the end of twenty-four hours than higher ratios. However, the number of pathological sera which were observed during the full twenty-four hour period were too few to permit correct interpretation of this finding.

#### SUMMARY

1. The colloid osmotic pressure of serum protein has been determined by a type of osmometer which permits observations of the pressure to be made at any desired interval of time, and which insures sterility of the serum.

2. Once the colloid osmotic pressure of serum having a normal concentration of protein was developed it was found to remain constant for sixteen to eighteen hours.

3. The colloid osmotic pressures of pathological sera with low concentrations of protein were found to be unstable. After rising to a maximum during the course of three to five hours the pressure began to fall gradually.

4. The reduced stability of the colloid osmotic pressure of hypoproteinemic sera was reproduced experimentally by dilution of normal serum with physiological saline or with the ultrafiltrate from normal serum.

5. Concentration of the proteins of hypoproteinemic sera to 6 per cent or above by ultrafiltration resulted in a colloid osmotic pressure curve indistinguishable from that of normal serum.

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# THE RELATIONSHIP OF THE BLOOD GLUCOSE TO THE CONCENTRATION OF LACTOSE IN THE MILK OF LACTATING DIABETIC WOMEN

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The mammary gland has a specific function, namely the secretion of milk. In the process of elaborating this unique fluid, the gland actually manufactures quantities of protein, fat and carbohydrate, as casein, milk fat, and lactose are the specific substances produced. Since these constituents are not found in blood normally, they are assumed to be synthesized by the mammary gland from the glucose, amino acids and the phospholipine brought to it by the blood (1). My interest in the problem is limited to the carbohydrate metabolism, and this paper deals with the presentation of such data. If, as is generally accepted, the glucose of the blood is the raw material from which lactose is made, the question arises whether there is a quantitative relationship between the two. Can the concentration of the lactose be increased or diminished by altering the glucose concentration of the blood?

There is considerable evidence obtained from animal experiments that the glucose of the blood is the precursor of the lactose of the milk. There are also on record perfusion experiments on excised mammary tissue of animals which lend support to this view. In addition, there are data derived from experiments which go a step further. These experiments demonstrate that not only is the lactose of the milk synthesized from the blood glucose, but that there is a quantitative relationship between them as regards the concentration and the total quantity produced.

In 1884 Bert (2) removed the mammary glands of goats. These animals were then bred and after parturition a reducing substance was found in the urine. The urine of the control animals did not contain this reducing substance. From such findings Bert made the following deductions: First, that a hyperglycemic state occurs after parturition to furnish the lactating gland with more material for the production of lactose, and second,

that since the mammary gland is not present to utilize the sugar a mellituria develops. The nature of this reducing substance found in the urines of such experimental animals was not known until 1909 when Porcher (3) confirmed Bert's work and identified the reducing substance as *glucose*. Both of these investigators stated that the postpartum glycosuria of their experimental animals was of short duration. These experiments furnished indirect evidence that the body prepares itself for the process of lactation by an elevation of the blood sugar and that it is the glucose of the blood which is used for the manufacture of the lactose. There are other animal experiments which have a bearing on the problem, and which more definitely establish such a relationship. Kaufmann and Magne (4) working with cows, took samples of blood from the mammary vein and also the jugular vein for glucose studies. The specimen from the jugular vein was assumed to be comparable in composition to that of the mammary artery. In lactating animals the blood going to the mammary gland as represented by the jugular blood had eighteen per cent more glucose than the blood leaving it, while in non-lactating animals the sugar concentration of the blood showed no such difference. Cary (5), cited by Meigs, confirmed these results. This investigator was extremely careful to obtain specimens simultaneously from the jugular and mammary veins by means of cannulae in each so that the time variable would be minimized.

The above experiments with living animals offer strong evidence that glucose is the precursor of the milk sugar. An additional clinical observation lends further support to this conclusion. It is recognized among dairy men that heavy milking cows develop a condition known as milk fever and that this condition may develop acutely during the process of milking. The clinical picture and



as alteration in pH of the serum during the period of equilibration, require further investigation in this connection.

It is evident that measurement of the colloid osmotic pressure at the end of twenty or twenty-four hours gives only an approximate idea of the total pressures which hypoproteinemic sera are capable of developing. In general, the lower the concentration of protein the less representative will be the twenty-four readings. A:G ratios appear to have had little or no effect upon the development of maximum pressures. On the other hand, ratios below one seem to have been more frequently associated with low pressures at the end of twenty-four hours than higher ratios. However, the number of pathological sera which were observed during the full twenty-four hour period were too few to permit correct interpretation of this finding.

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TABLE I

*Showing the constancy of the milk lactose after marked blood sugar elevations*

	Fast- ing	Following the injection of 100 grams of glucose			
		1 hour	1 hour	2 hours	3 hours
Patient J. G.					
Blood sugar, <i>mgn. per</i> <i>100 cc.</i> .....	221	313	341	384	358
Lactose of milk, <i>grams</i> <i>per 100 cc.</i> .....	8.0	7.5	8.0	8.2	7.8
Patient R. S.					
Blood sugar, <i>mgn. per</i> <i>100 cc.</i> .....	150	180	340	380	450
Lactose of milk, <i>grams</i> <i>per 100 cc.</i> .....	7.4	7.7	7.6	7.6	7.5
Patient E. K.					
Blood sugar, <i>mgn. per</i> <i>100 cc.</i> .....	102	206	213	238	189
Lactose of milk, <i>grams</i> <i>per 100 cc.</i> .....	6.9	6.2	5.6	5.8	5.0
Patient M. M.					
Blood sugar, <i>mgn. per</i> <i>100 cc.</i> .....	109	172	293	299	225
Lactose of milk, <i>grams</i> <i>per 100 cc.</i> .....	7.3	6.1	7.0	6.9	6.7
Patient H.					
Blood sugar, <i>mgn. per</i> <i>100 cc.</i> .....	104	160	244	290	191
Lactose of milk, <i>grams</i> <i>per 100 cc.</i> .....	5.3	5.2	5.5	5.6	5.4

TABLE II

*Showing the constancy of the milk lactose after marked lowering of the blood sugar*

	Fast- ing	
Patient J. G.		3½ hours after 30 units of insulin
Blood sugar, <i>mgn. per</i> <i>100 cc.</i> .....	177	85
Lactose of milk, <i>grams</i> <i>per 100 cc.</i> .....	7.9	7.9
Patient H.		3½ hours after 20 units of insulin
Blood sugar, <i>mgn. per</i> <i>100 cc.</i> .....	147	52
Lactose of milk, <i>grams</i> <i>per 100 cc.</i> .....	6.6	5.8
Patient R.		3½ hours after 20 units of insulin
Blood sugar, <i>mgn. per</i> <i>100 cc.</i> .....	119	44
Lactose of milk, <i>grams</i> <i>per 100 cc.</i> .....	6.3	6.5
Patient S.		3½ hours after 15 units of insulin
Blood sugar, <i>mgn. per</i> <i>100 cc.</i> .....	178	44
Lactose of milk, <i>grams</i> <i>per 100 cc.</i> .....	7.2	6.9

cose content of the blood. The results of the four experiments in which insulin was administered refute such objections.

It is now well established that in diabetes, insulin aids in the utilization of carbohydrates. In other words it converts, at least for the period of its activity, a diabetic into a non-diabetic. That being the case the four diabetic women, to whom insulin was given and whose blood sugar and milk lactose were studied, were for the period of the insulin activity normal, as regards their ability to utilize glucose. The sharp drop in the blood glucose following the administration of insulin support this inference. Yet, in spite of this pronounced fall in the blood glucose, three hours after the patient had received insulin, the concentration of the milk sugar was not lowered. As a matter of fact, it remained remarkably constant even though there was progressively less circulating glucose from which lactose was synthesized. This observation thus reveals that the lactating diabetic woman secretes milk, the lactose content of which does not differ from the normal, and furthermore, the concentration of the lactose is not influenced by variations in the blood glucose.

the laboratory evidence point to a hypoglycemic reaction. To remedy this condition measures are directed towards elevating the blood sugar. This has been done by either injecting glucose into the blood stream or by stopping the production of milk by inflating the udder with air. It has been inferred that the latter procedure is effective because by terminating lactation it enabled the organism to retain its glucose and thus maintain the concentration of this substance at a normal level in the blood and tissues.

Foa (6), working with excised mammary glands of sheep, demonstrated that not only is the blood glucose a precursor of the milk lactose, but that a quantitative relationship exists between them. He perfused excised glands kept in Ringer's solution, and he could definitely increase the lactose content of the milk produced by increasing the concentration of glucose in the perfusing fluid. He also noted that blood and Ringer's solution as the perfusing mixture caused a secretion of milk; that Ringer's solution and dextrose alone caused the secretion of a watery fluid containing lactose; that the glucose content of the perfusing fluid diminished as the breast continued to produce lactose, and furthermore that Ringer's solution alone or with galactose did not result in the formation of lactose. These experiments offer conclusive evidence that the excised mammary glands convert glucose to lactose, and that the more glucose in the perfusing fluid, the greater was the concentration of the lactose produced.

The application of such results to living animals might meet with objections because conditions are much altered, and furthermore the nervous control of the secretory mechanism is removed. It would, therefore, be desirable to produce a hyperglycemia in the living animal and note the resulting concentration of lactose from such a procedure. In the normal living animal Meigs (7) states that the concentration of milk sugar is most constant and is in no way affected by variations in the diet. This statement is not an unexpected finding as it is difficult to produce abnormal elevations in the glucose concentration in the blood of normal animals by increasing the carbohydrate content of the diet. Given, however, a lactating diabetic patient whose blood sugar can be elevated to markedly abnormal levels, or whose blood glucose can

also be dropped below the normal concentration, what effect will such procedure have on the lactose concentration of the milk?

In an attempt to answer this question five diabetic lactating mothers were employed. All were about two weeks postpartum. The severity of the diabetes varied but all were controlled without much difficulty while in the hospital. The control of their diabetes was somewhat more difficult while they were cared for in the outpatient department during the antenatal period, but at no time were they serious problems. One of the patients was managed by diet only. The other three required insulin to maintain the urine free from sugar. The maximum amount used in any case was 30 units, the minimum was 15 units. The youngest patient was 30 and the oldest 40 years old. The blood sugar was elevated by the administration of glucose by mouth. All of the patients were given 100 grams of glucose, before breakfast. Blood was drawn before and at  $\frac{1}{2}$ , 1, 2, and 3 hours following the ingestion of the glucose. The blood specimens were obtained from the antecubital vein and discharged into tubes containing about 2 mgm. of potassium oxalate for each cubic centimeter of blood. The breast milk was obtained at about the same time intervals by means of a breast pump. The first 2 or 3 cc. of milk collected at the times specified were discarded. The remainder was used for analysis.

In four of the patients, the blood glucose and lactose of the breast milk were studied following the administration of insulin. These patients reported before breakfast at which time a blood and milk sample were obtained. Following this, insulin was administered subcutaneously. The amount given varied in each case and depended on the height of fasting blood sugar. The exact amounts injected are shown in Table II. About  $3\frac{1}{2}$  hours later, when symptoms of nervousness, hunger and a feeling of heat became apparent, blood and milk samples were again obtained. It was felt that the clinical manifestations mentioned were early symptoms of a hypoglycemia. During this experimental period none of the patients received any food or fluids.

The glucose of the blood was determined by the colorimetric method of Benedict (8). The

lactose of the milk was done by the procedure of Owen and Gregg (9).

From Tables I and II it is obvious that the concentration of the milk sugar in the human lactating diabetic is constant, and is not influenced by the quantity of blood glucose circulating at a given time. This fact is not in accord with Foa's conclusions, but the difference might be easily accounted for by the difference in experimental conditions. The above data are based on results obtained on living diabetic women, while Foa's conclusions were drawn from results of perfusion experiments in which excised breasts of non-diabetic animals were used.

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TABLE II

*Showing the constancy of the milk lactose after marked lowering of the blood sugar*

	Fast- ing	
Patient J. G.		3½ hours after 30 units of insulin
Blood sugar, <i>mgm. per</i> <i>100 cc.</i> .....	277	85
Lactose of milk, <i>grams</i> <i>per 100 cc.</i> .....	7.9	7.9
Patient H.		3½ hours after 20 units of insulin
Blood sugar, <i>mgm. per</i> <i>100 cc.</i> .....	147	52
Lactose of milk, <i>grams</i> <i>per 100 cc.</i> .....	6.0	5.8
Patient R.		3½ hours after 20 units of insulin
Blood sugar, <i>mgm. per</i> <i>100 cc.</i> .....	119	44
Lactose of milk, <i>grams</i> <i>per 100 cc.</i> .....	6.3	6.5
Patient S.		3½ hours after 25 units of insulin
Blood sugar, <i>mgm. per</i> <i>100 cc.</i> .....	178	44
Lactose of milk, <i>grams</i> <i>per 100 cc.</i> .....	7.2	6.9

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## SUMMARY AND CONCLUSIONS

Five diabetic, lactating women were studied to determine whether a quantitative relationship exists between the concentration of glucose in the blood and of lactose in the milk. The blood sugar was elevated by means of glucose ingestion and lowered by varying doses of insulin. It was found in every instance that the concentration of lactose in the breast milk remained remarkably constant in spite of very marked elevations or depressions of the blood glucose concentration.

My thanks are due to Dr. Henricus J. Stander of the Department of Obstetrics and Gynecology for the opportunity of studying the cases. Mr. J. Francis Cadden performed the milk analyses. This is gratefully acknowledged.

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# EXPERIMENTAL BUNDLE BRANCH BLOCK IN THE MONKEY<sup>1</sup>

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(From the Dept. of Physiology and Pharmacology, Long Island College of Medicine, Brooklyn)

(Received for publication July 10, 1935)

Among the evidence upon which the electrocardiographic localization of bundle branch block depends is the experiment performed by Lewis on a Rhesus monkey. This result he considered to be crucial, and it formed in large part the basis from which he derived his views regarding the levocardiogram and dextrocardiogram (1). Since his findings are in direct conflict with so much other convincing evidence it was decided to repeat this work. It is the purpose of the present communication to present the electrocardiographic changes consequent to transection of the main branches of the Bundle of His in a series of Rhesus monkeys.

## METHOD

Twelve adult Rhesus monkeys were used, the anesthesia being ether by inhalation. German silver wire electrodes were inserted under the skin of the extremities. The chest was opened in the anterior axillary line on the left side following the institution of artificial respiration, and a vertical slit was made in the pericardium in order to expose the heart. Control electrocardiograms, using the three standard leads, were taken before and after the chest cavity was entered.

The branches of the Bundle of His were cut with a Knapp knife needle, the method used being a slight modification of that employed in a previous investigation (2). If electrocardiograms taken after the initial attempt remained normal, the knife was reinserted and the operation repeated. Following the production of the desired lesion, as manifested by electrocardiographic alterations, tracings were recorded at intervals to note the permanency of the change.

After one or the other branch was cut, premature contractions were produced by single induction shocks applied to the outer walls of the heart. At the completion of each experiment the

heart was examined and the position and extent of the cut observed. In most specimens the main branches could be seen macroscopically directly beneath the endocardium, and without difficulty the location of the section could be ascertained. For further confirmation histologic studies were made on two of the hearts.

## RESULTS

In two successful experiments the left branch was cut and in a similar number the right. In several other instances repeated attempts were necessary before successful transection of a division was accomplished. None of these was incorporated in the results because the electrocardiographic alterations were complicated by extensive injury to the myocardium or to the bundle itself. Also, none was used in which there was damage to the septum of the opposite ventricle. However, no data generally inconsistent with recorded results were obtained.

Of the two experiments in which the left branch was definitely cut, the subsequent electrocardiograms in both instances showed an initial main deflection in Lead I which was upwardly directed and widened with a terminal wave downwardly directed. Lead III presented a widened negative QRS complex with the terminal wave opposite in sign. The curves were therefore of the discordant type (in the sense employed by Lewis) (Figure 1).

In the two instances of successful transection of the right branch, the QRS complex was widened and downwardly directed in Lead I with a terminal upward wave. In Lead III the main deflection was upright in one instance and the reverse in the other, the terminal wave always being opposite to the main deflection. On transection of the right branch, therefore, the curves were either concordant or discordant, with the main deflection in Lead I being consistently negative (Figures 2 and 3).

<sup>1</sup> Read by title at the Proceedings of the American Society for Clinical Investigation, Atlantic City, May 6, 1935.

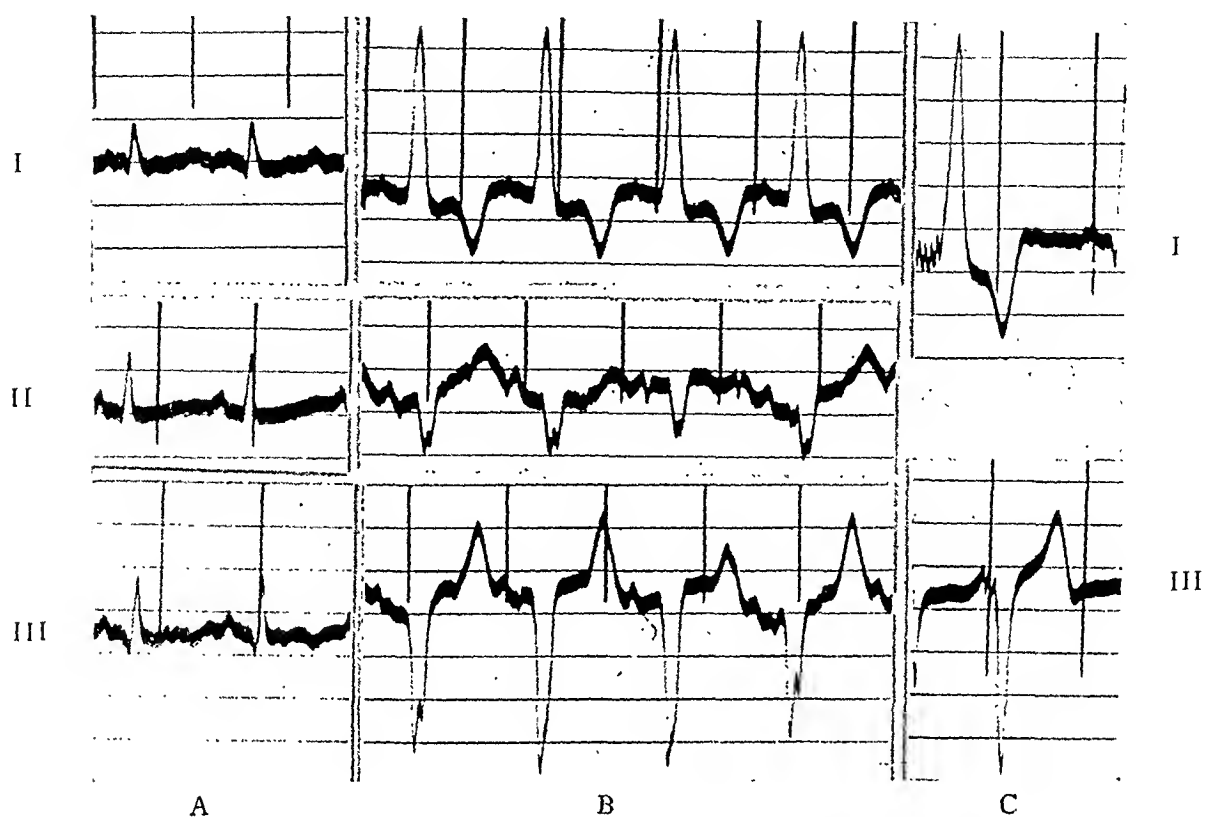


FIG. 1. TRANSECTION OF LEFT BRANCH OF BUNDLE OF HIS.

*A*, normal standard three leads. *B*, following transection, discordant type of curves produced. *C*, Leads I and III, showing extrasystolic waves produced by stimulation of outer wall of right ventricle. 1 cm. equals 1 millivolt. Time, 0.2 second.

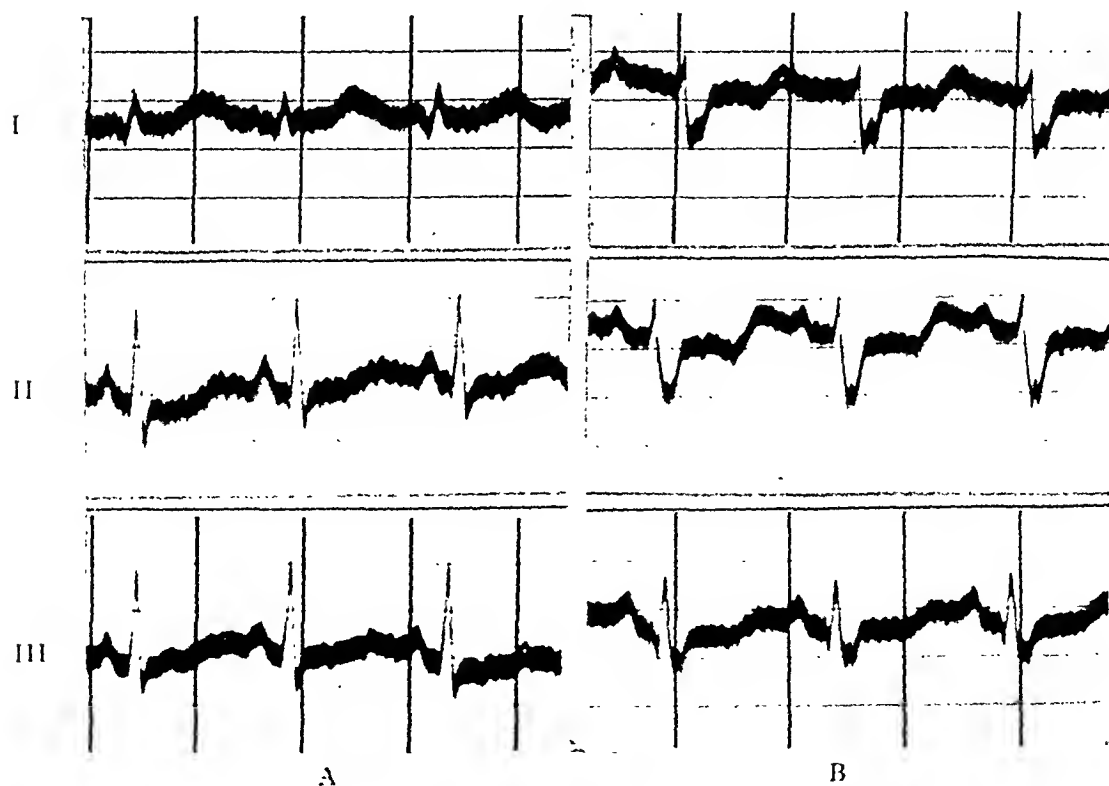


FIG. 2. TRANSECTION OF RIGHT BRANCH OF BUNDLE OF HIS.

*A*, normal standard three leads. *B*, following transection, discordant type of curves produced.

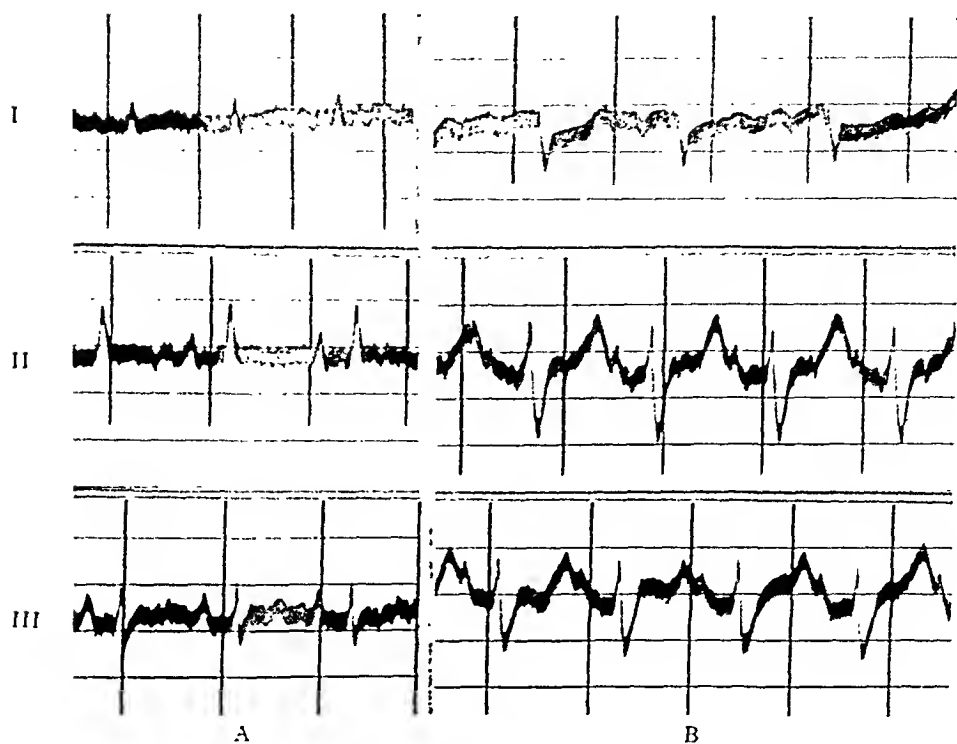


FIG. 3. TRANSECTION OF RIGHT BRANCH OF BUNDLE OF HIS.

*A*, normal standard three leads. *B*, following transection, concordant type of curves produced.

In the instances in which extrasystoles were obtained from one or the other ventricle, the results coincided with the above data. On stimulating the anterior wall of the right ventricle midway between apex and base, the initial main deflection in Lead I was upwardly directed (Figure 1), while a similar procedure applied to the left ventricle produced exactly opposite results. In other words, activation of the right ventricle before the left, either because of transection of the left main branch or direct stimulation of the right ventricle itself, affected Lead I so as to cause the main deflection to be positive. A corresponding relationship was found to be present also in the case of transection of the right branch and stimulation of the left ventricle.

#### COMMENT

The uncertainty concerning the electrocardiographic distinction between right and left bundle branch block still exists, although the new termi-

nology has come to be widely accepted. On the other hand, some authors have either adhered to the older viewpoint or else have stated that present data will not permit a definite decision.

In the effort to settle the difficulty a great deal of discussion and some very substantial evidence have been presented, all of which has been adequately reviewed in recent publications. In support of the classical terminology no experiment has been more widely cited than that of Lewis in which he compressed the right branch of the Bundle of His in a single Rhesus monkey. His original report does not include the actual records, but a chart representing the temporal and quantitative relationships of the initial ventricular deflections is illustrated (3). This chart shows an upwardly directed chief initial deflection in Lead I and a downwardly directed corresponding deflection in Lead III. Lewis called this the *levocardigram*, and from this data calculated a curve representative of the effects of left branch block in which the initial deflection is downward in



Lead I and upward in Lead III; this he called the dextrocardiogram.

So far as we know this experiment has been repeated but twice. Wilson and Hermann cut the right branch in one monkey but unfortunately obtained concordant curves (4). Subsequent transection of the left branch resulted in complete heart block. Since the completion of our experiments Kountz and his associates reported the effects of transection of the right branch in two monkeys and of the left in one (5). In the former, discordant curves were obtained which were down in Lead I and up in Lead III. In the latter, a curve was produced that was up in Lead I and down in Lead III. Their results and ours therefore are the direct opposite of those reported by Lewis.

#### SUMMARY

Following division of the left main branch of the His Bundle in the Rhesus monkey, the chief initial deflection of the electrocardiogram is up-right in Lead I and downward in Lead III.

Division of the right branch results in initial

deflections downwardly directed in Lead I and either upward or downward in Lead III.

Stimulation of the ventricle contralateral to the lesion produced complexes, the deflections of which corresponded in direction to those of the bundle branch block.

The recorded data are further evidence in support of the new terminology of bundle branch block localization.

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# DIETARY PROTEIN IN HEMORRHAGIC BRIGHT'S DISEASE.

## II. THE EFFECT OF DIET ON SERUM PROTEINS, PROTEINURIA AND TISSUE PROTEINS<sup>1</sup>

By E. HENRY KEUTMANN AND SAMUEL H. BASSETT

WITH THE TECHNICAL ASSISTANCE OF GERALDINE E. JULIAN, CLARA H. PRESENT  
AND HELEN E. VAN ALSTINE

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and Medical Clinic of the Strong Memorial and Rochester Municipal Hospitals,  
Rochester, New York)

(Received for publication July 10, 1935)

The comparatively few accurate studies of the nitrogen balance which have been made during chronic active stage of nephritis have been concerned chiefly with the quantitative aspect of protein requirement (17). Little attention has been given to the kind of protein fed and its effect upon hypoproteinemia, proteinuria and the storage of protein in the tissues.<sup>2</sup> The problem of hypoproteinemia is of especial interest, since no satisfactory means of increasing the concentration of the serum proteins of the nephritic has been discovered. Recently a systematic attempt has been made by Whipple and collaborators (10, 20) to ascertain factors governing the regeneration of plasma proteins. By repeated plasmapheresis of the dog they have shown the existence of a reserve depot containing precursors of the serum proteins. The quantity of protein released by the depot under the stimulus of depletion was often two or three times greater than the total amount of plasma protein originally present in the circulation. When the reserve had been exhausted the effect of different food proteins on the rate of regeneration was measured. The most satisfactory responses in order of decreasing potency were given by beef serum, smooth muscle, lactalbumin, boiled white of egg, skeletal muscle and liver. Owing to the excellent response of the experimental animal it seemed logical to test the effect of some of these

proteins on the hypoproteinemia of nephritis. The general scheme has been to superimpose the test protein on standard diets with the object of determining whether the ingestion of the supplementary protein would increase the concentration of the serum proteins, either directly by increasing the rate of regeneration, or indirectly by first repairing losses from the tissue proteins. In one case the experiment was continued for seven months in order that ample time might be provided for replacement of lost body protein, while in another instance the utilization of protein was studied at different levels of caloric intake.

### EXPERIMENTAL PROCEDURES

**Subjects.** The major part of the report is devoted to an investigation of two patients. The first was in the degenerative stage of chronic hemorrhagic Bright's disease. The second was a man whose illness was of shorter duration, and closely resembled a true nephrosis. Both patients were studied in the metabolism division of the hospital, where their diets were prepared and sampled in a manner previously described (1).

The third patient, also in the degenerative stage of chronic active hemorrhagic Bright's disease, was studied on the general medical division. His diets were prepared in the special diet kitchen of the hospital but were not sampled or analyzed.

**Diets.** The plan of varying the constituents of the diet differed with each subject, which necessitates a separate discussion of each case. In general the plan was to keep each patient on a control (basal) diet, from time to time superimposing the protein to be tested or additional calories in the form of carbohydrate and fat.

To provide some variety three menus were prepared for each diet. These varied slightly but had the same caloric and protein content, and were fed consecutively during each three day period. The patients thus ate the same food every third day. All of the diets were prepared without salt.

**Protein supplements.** With one exception, which is discussed under the case of L. R., the protein supplements

<sup>1</sup> Aided by a grant from the Fluid Research Fund contributed by the Rockefeller Foundation.

<sup>2</sup> A paper published by Liu and Chu (J. Clin. Invest., 1935, 14, 293) after the completion of this manuscript has come to our attention. Problems dealt with in their report are similar to some of those mentioned here. Since their findings do not appear to invalidate any of our observations the reader is referred to their article without further comment.

were superimposed on the basal diet, giving an increase in caloric intake corresponding to the amount of the supplement. The supplements were divided into three equal portions and given together with the meals.

*Lactalbumin* was obtained from the Harris Laboratories. Its nitrogen content as given by the manufacturer was confirmed by analysis.

*Serum protein* was prepared from the serum of cattle. That given to the first patient was precipitated by the addition of one and a half volumes of alcohol, that given to the second patient by coagulation with heat at 80° C. In each case the precipitate was washed with 95 per cent alcohol, dried in a vacuum oven at 50° C., and reduced to a powder in a ball mill.

*Liver protein* was given in the form of the dried commercial residue remaining after the factors potent in the treatment of primary and secondary anemias had been extracted from fresh liver. Before feeding, the material was treated with hot alcohol in a Bloor extractor for about 48 hours, thus removing some pigments and rancid fats which gave the unextracted product an unpleasant odor and taste. The purified and dried residue, containing about 82 per cent protein, was reduced to a fine powder in a ball mill.

*Egg white.* The quantity of boiled egg white required to supply the desired amount of protein was computed from data published by Rose (21), and this amount was fed daily with the basal diet. The supplementary feedings were sampled with the other items of food and the difference between the average total nitrogen intake of these periods and the basal nitrogen intake was taken to be the amount in the supplement.

*Methods.* The nitrogen content of the diets, the stools and the daily urine was determined by a macro-Kjeldahl method (19). The protein content of the diets was computed as diet nitrogen  $\times$  6.25, no allowance being made for the presence of nitrogen extractives in the food. Because of the many steps involved in preparing, sampling and analysing, it was found that the most reliable estimate of the amount of nitrogen eaten in each period was derived by taking the mean of all analyses for the diet concerned. The protein of the urine was determined by the micro-Kjeldahl method described by Peters and Van Slyke (19). Serum proteins were determined by the method of Howe (19). Blood for these determinations was drawn without stasis in the morning, about fourteen hours after the previous meal, and while the patients were still recumbent. Plasma volumes were determined by the dye method of Keith, Rowntree and Geraghty (12) as modified by Hooper, Smith, Belt and Whipple (11). The patients were weighed at the same time each morning after emptying the bladder and prior to the ingestion of food. The stools were separated by means of carmine given at the beginning of each metabolic period (1).

*Calculation of nitrogen and protein balances.* Peters and Bulger (17) have shown that it is impossible to obtain an accurate estimate of the protein balance without

taking account of the changes in concentration of non-protein nitrogen in the body fluids.

The following example is cited to illustrate the method: In the first patient the greatest change in nonprotein nitrogen took place during Period 9. At the end of Period 8 the nonprotein nitrogen of the patient's blood was 32 mgm. per 100 cc. and he weighed 69.7 kilograms. The nonprotein nitrogen stored in his body was estimated as being  $69.7 \times 0.7 \times 0.32 = 15.6$  grams.<sup>3</sup> During Period 9 the protein in his diet was increased and at the end of the three day period the blood nonprotein nitrogen was 52 mgm. per 100 cc. His weight being 69.8 kilograms, the nonprotein nitrogen content of the body was estimated as being  $69.8 \times 0.7 \times 0.52 = 25.4$  grams. Therefore, the nonprotein nitrogen content of the body was estimated to have increased 9.8 grams during this period.

The balance of the calculations were as follows:

Intake .....	85.70 grams
Urine N.P.N. ....	38.6 grams
Stool N <sup>4</sup> .....	3.66 grams
Increase of body N.P.N. ..	9.8 grams
<hr/>	
N from catabolized protein	52.06 grams
N lost as urine protein ....	7.9 grams
<hr/>	
	59.96 grams
Subtracting this sum from the intake ....	59.96 grams
<hr/>	
N deposited as protein .....	25.74 grams
N of urine protein .....	7.9 grams
<hr/>	
N equivalent to total amount of protein synthesized by the body .....	33.64 grams

The term "total protein synthesized" has been used to include the protein deposited and protein lost in the urine. The latter was either made from the diet or withdrawn from the protein stores of the body. In either case the ability of the body to recapture food protein was partly expended in counterbalancing proteinuria.

The same method of computation was used when there was a decrease of body protein either because of catabolism or proteinuria. In such instances a negative value is found in the columns designated "deposited protein." (Tables I and IV, Figures 1 and 2.)

<sup>3</sup> This approximation may be made by assuming that the body water constitutes 70 per cent of the body weight, its nonprotein nitrogen concentration being the same as that found in the blood. Edema fluid, when present, was considered to contain 90 per cent water (17).

<sup>4</sup> The consideration of all of the nitrogen of the stools as catabolic is probably not correct in all instances. For calculating the biological values of proteins the method of Mitchell (15) which involves the deduction of unabsorbed nitrogen from the intake is more accurate. In the present instance the deduction of unabsorbed nitrogen from the intake is not necessary since the purpose of the experiment was to determine the utilization of the foods as eaten.

A slight error was probably involved in the correction for changes in the stores of nonprotein nitrogen, and for this reason periods which came at the time of such readjustment were never used as controls. For the most part, the calculations of nitrogen balances were not complicated by the presence of edema.

#### PRESENTATION OF DATA

*Case Number 1.* Degenerative stage of chronic hemorrhagic Bright's disease. L. R. was a 25 year old accountant who developed edema in April, 1933, eight or ten days after a severe upper respiratory infection. He was found to have proteinuria, hematuria, and cylindruria. The edema disappeared on restricting salt, but general malaise persisted until entrance to the hospital on July 31, 1933.

Physical examination at that time showed a well developed man. There was no edema. The eyegrounds were normal. The systolic blood pressure was 120 mm. Hg, the diastolic was 75 mm. Hg. Purulent material could be expressed from the left tonsil. Other foci of infection were not found. Tonsillectomy was performed on August 8th, and revealed bilateral tonsillar abscesses from which hemolytic streptococci were grown. Recovery from the operation was uneventful, and at discharge he was advised to take a diet containing approximately 100 grams of protein and low in salt.

He returned for observation several times during the summer, and on October 6th, 1933 was admitted to the metabolism division of the hospital for study. The physical examination at this time revealed evidence of very slight edema of the ankles which disappeared during the first few days on a salt free diet. The systolic and diastolic blood pressures were 115 and 75 mm. Hg respectively.

During the seven months of residence in the metabolism division he remained in excellent spirits and improved physically, but there was little change in the status of his renal disease. In April, a repetition of the roentgenograms of the teeth suggested periapical absorption around a lower right molar which was extracted on April 24th. Temporary increase in albuminuria followed this procedure as indicated in Table I, Period 67. Cultures of the extracted tooth yielded only diphtheroids.

*Laboratory findings:* Urine: August 3, 1933. About 10 grams protein in 24 hours. Addis sediment counts:

	Formed elements in millions per 12 hours		
	R.B.C.	Epithelial and W.B.C.	Casts
Aug. 3, 1933.....	18	29	7.4
Sept. 19, 1933.....	28	35	1.3
Dec. 28, 1933.....	13	8	.75
Apr. 26, 1934.....	15	12	1.2
May 15, 1934.....	9.5	10	.6

#### Urea clearance tests:

	per cent
Aug. 2, 1933	42
Sept. 22, 1933	47
Oct. 18, 1933	43
Nov. 15, 1933	64 (high protein diet)
Dec. 29, 1933	60 (high protein diet)
May 14, 1934	48

#### Blood hemoglobin content:

	grams per 100 cc.
July 31, 1933	12
Oct. 20, 1933	13.4
Jan. 9, 1934	13.3
Mar. 10, 1934	13.5

*Serum protein:* Aug. 1, 1933, 4.3 per cent (colorimetric method); September 19, 1933, 4.2 per cent (colorimetric method). Subsequent determinations (done by the method of Howe) are recorded in Table II.

The blood Wasserman reaction, blood sugar, blood non-protein nitrogen, and CO<sub>2</sub> capacity of the serum were normal. A culture of the blood remained sterile.

*Diet.* The daily basal diet given in all periods except numbers 9 to 27 inclusive (Table I and Figure 1) contained 399 grams of carbohydrate, 119 grams of fat and 67.5 grams of protein, with an energy value of 3020 calories. The sources of protein (in grams) were as follows: Vegetable, 24.3; milk, 11.6; egg, 8.1; meat, 23.5; total, 67.5. Of the meat protein 55 per cent was derived from ground beef steak, 28 per cent from ground veal steak and 17 per cent from tuna fish. Fluid intake was fixed at 2500 ml.

In Periods 9 to 27 inclusive the patient received a high protein diet containing 249 grams of carbohydrate, 134 grams of fat and 185 grams of protein, giving a caloric intake of 3030.

The protein (in grams) came from the following sources: Vegetable, 25.9; milk, 42.9; egg, 37.1; meat, 79.1; total, 185. The meat fraction consisted of 79 per cent ground beef steak and 21 per cent ground veal steak.

Following Period 27 there was a series of periods during which the patient received the basal diet plus various protein supplements. These supplementary periods were alternated with a series of control periods when the basal diet alone was given. The exact order in which these followed each other is indicated in Table I and Figure 1.

*Explanation of tables.* In Table I and Figure 1 are given the significant data from the nitrogen balances. Periods in which an obvious change in the metabolism of nitrogen was taking place have been listed separately, while those in which conditions were found to be essentially constant have

TABLE I  
Case 1, L.R.—Protein metabolism

Period	Protein intake	Calories per diem	Weight	Protein metabolism				
				In- take	Catabolized protein	Urine protein	Deposited protein	Total protein synthe- sized
<i>3 days each</i>	<i>grams per diem</i>		<i>kgm.</i>	<i>grams N per period</i>	<i>grams N per period</i>	<i>grams N per period</i>	<i>grams N per period</i>	<i>grams N per period</i>
1.....	Basal diet 70	3020	68.6	33.83	21.65	4.82	7.21	12.18
2 to 8...			67.9	33.83	21.58	5.27	6.71	12.25
9.....	Diet 180	3030	69.7	33.83	21.58	5.27	6.71	12.25
10.....			69.8	85.70	52.06	7.90	25.74	33.64
11.....			70.1	85.70	61.32	7.35	17.03	24.38
12.....			70.0	85.70	63.69	7.26	14.75	22.01
13 to 27..			70.5	85.70	66.93	7.03	11.74	18.77
			73.3	85.70	70.90	6.09	8.71	14.80
28.....	Basal diet 70, lactalbumin 50	3225	73.7	56.13	45.30	5.03	5.80	10.83
29, 30...			74.7	56.13	44.87	5.07	6.19	11.26
31, 32...	Basal diet 70, lactalbumin 100	3430	75.4	78.43	60.84	6.54	11.05	17.59
33, 34...			75.6	78.43	63.26	6.76	8.41	15.17
35, 36...	Basal diet 70	3020	76.2	33.83	30.24	5.31	— 1.72	3.59
37.....			75.7	33.83	29.42	5.18	— 0.77	4.41
38.....			75.8	33.83	27.34	5.16	1.33	6.49
39.....	Basal diet 70, egg white (cooked) 45	3005	75.9	54.87	34.92	6.15	13.80	19.95
40.....			75.4	54.87	38.10	7.70	9.07	16.77
41, 42...			75.8	54.87	39.90	7.47	7.50	14.97
43, 44...		3200	77.1	54.87	40.72	6.58	7.57	14.15
45 to 47..	Basal diet 70, egg white 23	3100	75.9	44.71	31.90	5.68	7.13	12.81
48.....	Basal diet 70	3020	76.2	33.83	26.65	5.65	1.53	7.18
49, 50...			76.0	33.83	26.51	5.74	1.58	7.32
51.....	Basal diet 70 Serum protein 10	3060	76.0	38.63	28.91	4.91	4.81	9.72
52.....			75.9	38.63	28.68	6.22	3.73	9.95
53.....			75.8	38.63	28.87	6.10	3.66	9.76
54.....	Basal diet 70 Serum protein 25	3120	76.0	45.83	32.73	6.54	6.56	13.10
55.....			75.7	45.83	35.81	6.26	3.76	10.02
56.....			75.8	45.83	36.10	6.17	3.56	9.73
57.....	Basal diet 70 Serum protein 50	3225	75.7	57.83	42.81	6.82	8.20	15.02
58.....			75.9	57.83	47.43	6.10	4.30	10.40
59.....			76.1	57.83	49.06	5.97	2.80	8.77
60 to 62..	Basal diet 70	3020	75.2	33.83	29.43	5.30	— 0.90	4.40
63.....			75.2	33.83	28.75	4.59	0.49	5.08
64.....	Basal diet 70 Liver residue protein 50	3225	75.6	57.83	41.80	6.18	9.85	16.03
65.....			75.0	57.83	44.79	7.64	5.40	13.04
66.....			75.0	57.83	46.49	7.54	3.80	11.34
67.....			74.7	57.83	46.47	10.04	1.32	11.36
68, 69...	Basal diet 70	3000	75.5	33.83	29.60	5.63	— 1.40	4.23
70, 71...			75.3	33.83	28.02	4.54	1.27	5.81

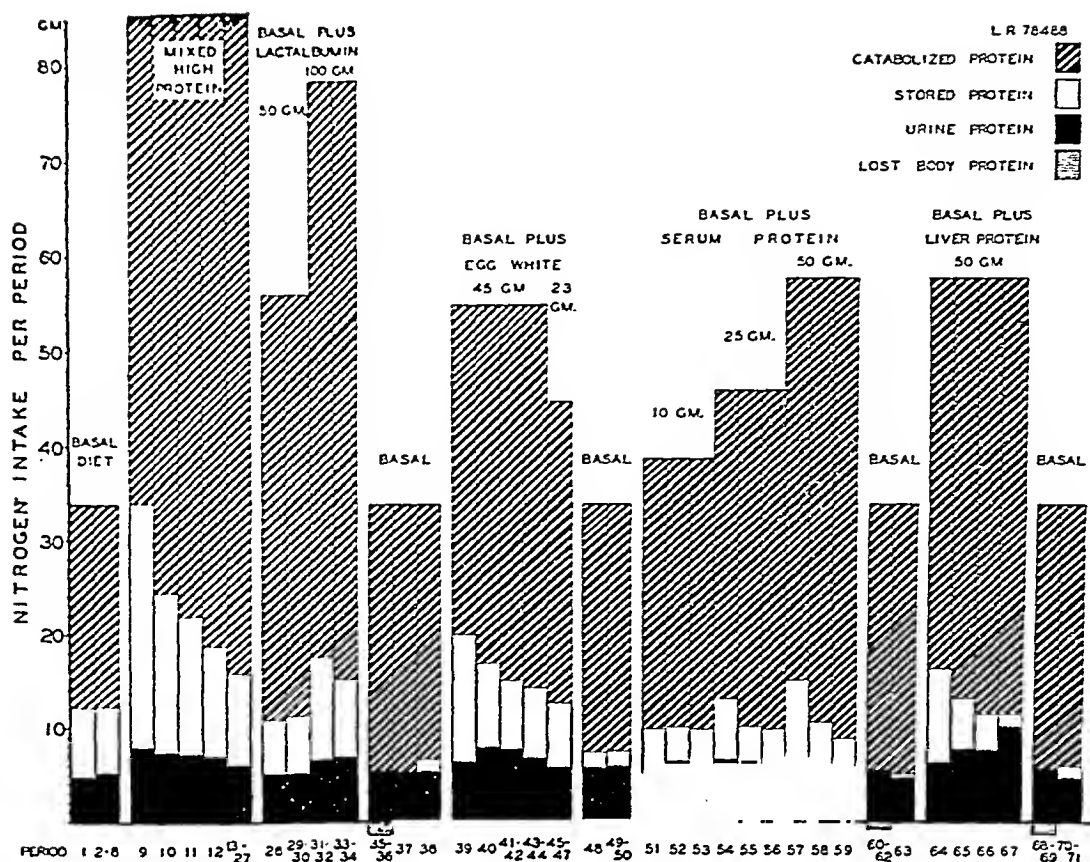


FIG. 1. CASE I, L. R.  
PROTEIN METABOLISM.

been grouped together, and average values have been recorded.

In Table II a comparison has been made of the utilization of the various proteins or mixtures of proteins. The percentage utilization of the total amount of protein ingested was computed as follows: Average nitrogen intake, Periods 1 to 8, 33.83 grams; average protein nitrogen in urine, 5.21 grams; average nitrogen deposited as protein, 7.03 grams; nitrogen converted into protein  $(5.21 + 7.03)$ , 12.24 grams;  $12.24 \div 33.83 \times 100 = 36.2$  per cent of the intake reconverted into protein.

As an illustration of the computation of the utilization of a supplement, Periods 39 to 42 in which 44 grams of egg white were fed daily may be taken. Average nitrogen content of the supplement, 21.04 grams; average nitrogen of total protein synthesized, 16.67 grams; average nitrogen synthesized to protein in proximate basal periods

(38, 49, 50) 7.04 grams. The nitrogen of the supplement converted into protein is estimated as  $(16.67 - 7.04) = 9.63$  grams. Percentage of nitrogen of egg white resynthesized to protein is  $9.63 \div 21.04 \times 100 = 45.7$  per cent.

### Protein metabolism

*Basal periods.* During the first series of periods on the basal diet the patient deposited protein and lost protein in his urine at a fairly constant rate as indicated in Table I and Figure 1. In these twenty-four days he lost 260 grams of protein in his urine, in spite of which his body gained 338 grams of protein. The total amount of protein formed in each period was about 36 per cent of his intake as shown in Table II. During subsequent basal periods less protein was synthesized. The main decrease was in the amount deposited, while, except for the effect of lag, the amount

TABLE I  
Case 1, L.R.—Protein metabolism

Period	Protein intake	Calories per diem	Weight	Protein metabolism				
				In- take	Catabolized protein	Urine protein	Deposited protein	Total protein synthe- sized
<i>8 days each</i>	<i>grams per diem</i>		<i>kgm.</i>	<i>grams N per period</i>	<i>grams N per period</i>	<i>grams N per period</i>	<i>grams N per period</i>	<i>grams N per period</i>
1.....	Basal diet 70	3020	68.6	33.83	21.65	4.82	7.21	12.18
2 to 8...			67.9	33.83	21.58	5.27	6.71	12.25
9.....	Diet 180	3030	69.7	33.83	21.58	5.27	6.71	12.25
10.....			69.8	85.70	52.06	7.90	25.74	33.64
11.....			70.1	85.70	61.32	7.35	17.03	24.38
12.....			70.0	85.70	63.69	7.26	14.75	22.01
13 to 27..			70.5	85.70	66.93	7.03	11.74	18.77
			73.3	85.70	70.90	6.09	8.71	14.80
28.....	Basal diet 70, lactalbumin 50	3225	73.7	56.13	45.30	5.03	5.80	10.83
29, 30...			74.7	56.13	44.87	5.07	6.19	11.26
31, 32...	Basal diet 70, lactalbumin 100	3430	75.4	78.43	60.84	6.54	11.05	17.59
33, 34...			75.6	78.43	63.26	6.76	8.41	15.17
35, 36...	Basal diet 70	3020	76.2	33.83	30.24	5.31	— 1.72	3.59
37.....			75.7	33.83	29.42	5.18	— 0.77	4.41
38.....			75.8	33.83	27.34	5.16	1.33	6.49
39.....	Basal diet 70, egg white (cooked) 45	3005	75.9	54.87	34.92	6.15	13.80	19.95
40.....			75.4	54.87	38.10	7.70	9.07	16.77
41, 42...			75.8	54.87	39.90	7.47	7.50	14.97
43, 44...		3200	77.1	54.87	40.72	6.58	7.57	14.15
45 to 47..	Basal diet 70, egg white 23	3100	75.9	44.71	31.90	5.68	7.13	12.81
48.....	Basal diet 70	3020	76.2	33.83	26.65	5.65	1.53	7.18
49, 50...			76.0	33.83	26.51	5.74	1.58	7.32
51.....	Basal diet 70 Serum protein 10	3060	76.0	38.63	28.91	4.91	4.81	9.72
52.....			75.9	38.63	28.68	6.22	3.73	9.95
53.....			75.8	38.63	28.87	6.10	3.66	9.76
54.....	Basal diet 70 Serum protein 25	3120	76.0	45.83	32.73	6.54	6.56	13.10
55.....			75.7	45.83	35.81	6.26	3.76	10.02
56.....			75.8	45.83	36.10	6.17	3.56	9.73
57.....	Basal diet 70 Serum protein 50	3225	75.7	57.83	42.81	6.82	8.20	15.02
58.....			75.9	57.83	47.43	6.10	4.30	10.40
59.....			76.1	57.83	49.06	5.97	2.80	8.77
60 to 62..	Basal diet 70	3020	75.2	33.83	29.43	5.30	— 0.90	4.40
63.....			75.2	33.83	28.75	4.59	0.49	5.08
64.....	Basal diet 70 Liver residue protein 50	3225	75.6	57.83	41.80	6.18	9.85	16.03
65.....			75.0	57.83	44.79	7.64	5.40	13.04
66.....			75.0	57.83	46.49	7.54	3.80	11.34
67.....			74.7	57.83	46.47	10.04	1.32	11.36
68, 69...	Basal diet 70	3000	75.5	33.83	29.60	5.63	— 1.40	4.23
70, 71...			75.3	33.83	28.02	4.54	1.27	5.81

*Lactalbumin.* Although there was considerable difference in the absolute amount of protein synthesized when lactalbumin was fed in quantities of 50 and 100 grams (Table I and Figure 1), it will be noted from Table II that there was no difference in the percentage of utilization at the two levels. This is of considerable interest in view of the entirely different result obtained with egg albumin and serum protein. Unfortunately, the comparison of these supplements is open to some criticism since control periods did not precede the ingestion of lactalbumin. However, the percentage utilization of the total protein intake was the same at both levels, and furthermore, the results are still the same when the last two periods at each level are compared. The effects of lag in nitrogen and protein excretion, which might have given an erroneous result in the first two periods (28 and 31) are thus excluded.

*Egg white.* Cooked egg white proved to be a very efficient protein for the manufacture of serum and tissue protein as is indicated in Tables I and II. Better utilization was again noted in the earlier than in the later periods.

A slight variation in the usual method of administration was adopted in this group. During Periods 39 to 42 inclusive, enough carbohydrate and fat was withdrawn from the basal diet to offset the extra calories furnished by the supplement, so that the caloric intake was kept at the basal level. In Periods 43 and 44 these 200 calories were again added to the diet. By comparing Periods 41 and 42 with 43 and 44 in Table I it may be noted that there was no change in the utilization. It is possible that the difference in caloric intake was not sufficiently large, but it is more likely that with a sufficiently high intake of carbohydrate and fat a maximum efficiency of utilization of protein is obtained which cannot be improved upon by an additional supply of nonprotein calories. Von Hoesslin's (23) data on the nitrogen balances of malnourished individuals are in agreement with this observation.

Comparison of proximate Periods 43 and 44 with 45 to 47 demonstrates that 23 gram supplements of egg white were more efficiently utilized than those of 44 grams (Table II).

*Serum protein* was added to the basal diet in three increments of 10, 25 and 50 grams daily.

With the addition of 10 grams, utilization was the same for the three periods, 51, 52 and 53 (Table I and Figure 1). With the 25 grams and 50 gram additions, the percentage of utilization declined from period to period.

The utilization at the different levels of intake, given in Table II, is again of interest. A definitely better utilization was obtained when the smaller supplement was given, as was the case with cooked egg white.

*Protein of liver residue.* Successive periods had the same decrease in degree of utilization as in the case of other substances previously given as supplements. Proteinuria was greatly increased in Period 67, but apparently this was not the result of increased formation of serum protein. On the last day of Period 66 a tooth had been drawn. Although it was thought to be infected, cultures of the root failed to demonstrate the presence of pathogenic micro-organisms. On the day of extraction and for three days following, there was a marked increase in proteinuria, probably due to a transient increase in the permeability of the glomerular capillaries.

Thirty-one per cent of the liver protein supplement was utilized for building protein. This was not as good utilization as that observed when egg white was fed, yet it was better than the utilization of beef serum protein or lactalbumin.

*Absorption of supplements.* The average nitrogen content of the stools in basal periods was 4 grams, and during the feeding of liver protein 8.13 grams. If the increase in fecal nitrogen was due to non-absorption of a portion of the supplement, then the available supplementary nitrogen was actually 4.13 grams less per period than estimated. Introduction of this correction would make the utilization of the "absorbed portion" of the liver residue 37 per cent. The amounts of egg white, serum protein and lactalbumin which remained unabsorbed were insignificant. Corrections for non-absorption of these proteins changed the values given for their utilization less than 1 per cent.

*Blood nonprotein nitrogen, serum proteins, and proteinuria.* The direction and extent of change in blood nonprotein nitrogen, serum proteins, and proteinuria are given in Table III. The level of blood nonprotein nitrogen at the end of each group



TABLE II

*Case 1, L.R.—Utilization of dietary and supplementary proteins*

Description of periods	Calories per kgm. per day	Per cent of total calories furnished by protein	Average nitrogen of food	Average nitrogen of supplement	Average total nitrogen intake	Average nitrogen synthesized into protein	Per cent of intake synthesized into protein	Per cent of supplement synthesized into protein
		<i>per cent</i>	<i>grams per period</i>	<i>grams per period</i>	<i>grams per period</i>	<i>grams per period</i>	<i>per cent</i>	<i>per cent</i>
Basal (1 to 8).....	44	9	33.83		33.83	12.24	36	
Mixed high protein (9 to 12).....	43	25	85.70	51.87	85.70	24.78	29	24
Mixed high protein (13 to 27).....	43	25	85.70	51.87	85.70	14.80	17	
Basal (38, 49, 50).....	40	9	33.83		33.83	7.04	21	
Lactalbumin, 50 grams per diem (28 to 30).....	43	15	33.83	22.3	56.13	11.12	20	18
Lactalbumin, 100 grams per diem (31 to 34).....	45	20	33.83	44.6	78.43	16.18	21	20
Basal (38, 49, 50).....	40	9	33.83		33.83	7.04	21	
Egg white, 44 grams per diem (39 to 42).....	40	15	33.83	21.04	54.87	16.67	30	46
Egg white, 44 grams per diem (43 to 44).....	42	15	33.83	21.04	54.87	14.15	26	34
Egg white, 23 grams per diem (45 to 47).....	41	12	33.83	10.88	44.71	12.81	29	53
Basal (49, 50, 63).....	40	9	33.83		33.83	6.57	19	
Serum protein, 10 grams per diem (51 to 53).....	40	10	33.83	4.8	38.63	9.81	25	68
Serum protein, 25 grams per diem (54 to 56).....	41	12	33.83	12.0	45.83	10.95	24	37
Serum protein, 50 grams per diem (57 to 59).....	42	15	33.83	24.0	57.83	11.40	20	20
Basal (63, 70, 71).....	40	9	33.83		33.83	5.54	16	
Liver residue protein, 50 grams per diem (64 to 67).....	42	15	33.83	24.0	57.83	12.94	22	31

of protein lost in the urine remained the same as in earlier basal periods.

This behavior was probably due to the fact that in the beginning the undersaturation of the tissue depots with protein permitted those reactions concerned with the formation of protein to proceed more rapidly than the reactions leading to its breakdown. During the later basal periods the tissues seem to have been more nearly saturated, and an equilibrium between incoming building stones and outgoing end products was approached.

*Mixed high protein diet.* Ingestion of the high protein diet led to deposition of considerably larger quantities of protein and the loss of more protein in the urine. In the first period of this diet (Period 9) about 39 per cent of the intake was utilized in formation of body protein. This was about the same proportion that was utilized with the basal diet; however, it must be pointed out that the correction for stored nonprotein nitrogen,

which was calculated as 9.8 grams, may have been too small, and this would make the calculation of 39 per cent too high. In the second period which was not subject to this possibility of error, 28 per cent was utilized. Thereafter, there was a decrease in utilization for several periods until deposition and loss in the urine took place at a fairly even rate (Periods 13 to 27).

The fact that the urinary protein as well as the amount deposited increased suggests a greater formation of both tissue and plasma proteins. The interesting, as well as disconcerting thing, is that in spite of this increased rate of manufacture and the deposition of 1150 grams of protein in 81 days, there was no apparent change in the total amount of serum protein nor in its concentration (Table III). In fact, serum proteins appear to have been lost from the circulation as rapidly as they were supplied to it from the depots or places of manufacture.

*Lactalbumin.* Although there was considerable difference in the absolute amount of protein synthesized when lactalbumin was fed in quantities of 50 and 100 grams (Table I and Figure 1), it will be noted from Table II that there was no difference in the percentage of utilization at the two levels. This is of considerable interest in view of the entirely different result obtained with egg albumin and serum protein. Unfortunately, the comparison of these supplements is open to some criticism since control periods did not precede the ingestion of lactalbumin. However, the percentage utilization of the total protein intake was the same at both levels, and furthermore, the results are still the same when the last two periods at each level are compared. The effects of lag in nitrogen and protein excretion, which might have given an erroneous result in the first two periods (28 and 31) are thus excluded.

*Egg white.* Cooked egg white proved to be a very efficient protein for the manufacture of serum and tissue protein as is indicated in Tables I and II. Better utilization was again noted in the earlier than in the later periods.

A slight variation in the usual method of administration was adopted in this group. During Periods 39 to 42 inclusive, enough carbohydrate and fat was withdrawn from the basal diet to offset the extra calories furnished by the supplement, so that the caloric intake was kept at the basal level. In Periods 43 and 44 these 200 calories were again added to the diet. By comparing Periods 41 and 42 with 43 and 44 in Table I it may be noted that there was no change in the utilization. It is possible that the difference in caloric intake was not sufficiently large, but it is more likely that with a sufficiently high intake of carbohydrate and fat a maximum efficiency of utilization of protein is obtained which cannot be improved upon by an additional supply of nonprotein calories. Von Hoesslin's (23) data on the nitrogen balances of malnourished individuals are in agreement with this observation.

Comparison of proximate Periods 43 and 44 with 45 to 47 demonstrates that 23 gram supplements of egg white were more efficiently utilized than those of 44 grams (Table II).

*Serum protein* was added to the basal diet in three increments of 10, 25 and 50 grams daily.

With the addition of 10 grams, utilization was the same for the three periods, 51, 52 and 53 (Table I and Figure 1). With the 25 grams and 50 gram additions, the percentage of utilization declined from period to period.

The utilization at the different levels of intake, given in Table II, is again of interest. A definitely better utilization was obtained when the smaller supplement was given, as was the case with cooked egg white.

*Protein of liver residue.* Successive periods had the same decrease in degree of utilization as in the case of other substances previously given as supplements. Proteinuria was greatly increased in Period 67, but apparently this was not the result of increased formation of serum protein. On the last day of Period 66 a tooth had been drawn. Although it was thought to be infected, cultures of the root failed to demonstrate the presence of pathogenic micro-organisms. On the day of extraction and for three days following, there was a marked increase in proteinuria, probably due to a transient increase in the permeability of the glomerular capillaries.

Thirty-one per cent of the liver protein supplement was utilized for building protein. This was not as good utilization as that observed when egg white was fed, yet it was better than the utilization of beef serum protein or lactalbumin.

*Absorption of supplements.* The average nitrogen content of the stools in basal periods was 4 grams, and during the feeding of liver protein 8.13 grams. If the increase in fecal nitrogen was due to non-absorption of a portion of the supplement, then the available supplementary nitrogen was actually 4.13 grams less per period than estimated. Introduction of this correction would make the utilization of the "absorbed portion" of the liver residue 37 per cent. The amounts of egg white, serum protein and lactalbumin which remained unabsorbed were insignificant. Corrections for non-absorption of these proteins changed the values given for their utilization less than 1 per cent.

*Blood nonprotein nitrogen, serum proteins, and proteinuria.* The direction and extent of change in blood nonprotein nitrogen, serum proteins, and proteinuria are given in Table III. The level of blood nonprotein nitrogen at the end of each group

TABLE III  
Case 1, L.R.

Period	Diet	Blood non-protein nitrogen *	Serum proteins *		Total circulating serum protein *	Urine protein	
			Total	A : G ratio		Total	A : G ratio
<i>5 days each</i>		<i>mgm. per cent</i> 33	<i>per cent</i>		<i>grams</i> (Period 4)	<i>grams per period</i>	
1 to 8.....	Basal	32	4.06	1.1	105	32.6	3.7
9 to 27.....	High protein	50	4.0	1.9	124	39.6	2.7
28 to 30.....	Lactalbumin, 50 grams per day	32	3.95	1.4	124	31.6	3.1
31 to 34.....	Lactalbumin, 100 grams per day	45	3.85	1.6	128	41.6	2.9
35 to 38.....	Basal	29	3.99	2.2	121	33.7	3.6
39 to 44.....	Egg white, 44 grams per day	38	3.9	1.8	119	43.7	7.0
45 to 47.....	Egg white, 23 grams per day	30	3.89		116	35.5	7.5
48 to 50.....	Basal	27	3.86	1.7	114	35.7	4.6
51 to 59.....	Serum protein	40	3.80	1.2	135	39.3	7.0
60 to 63.....	Basal	29	3.85	1.1	116	32.0	8.3
64 to 67.....	Liver protein	40	3.54	1.4	114	49.1	5.5
68 to 71.....	Basal	25	3.69	1.3		31.8	6.5
6 months after discharge.....		35	3.9	2.2			

\* Blood nonprotein nitrogen and serum protein determinations were made at end of each group of periods.

of periods was roughly parallel to the protein intake. Not all of the determinations which were made for the purpose of calculating the protein metabolism have been recorded in the table.

The concentration of serum proteins, as well as the total amount in circulation, remained nearly constant, and was uninfluenced either by giving large amounts of mixed proteins or by adding specific protein supplements to a basal diet. Withdrawals of blood for the first four determinations of serum proteins were made while the patient was semi-recumbent in bed. At that time the influence of posture (25) was not fully realized, so it is probable that the concentration of protein is slightly above the true basal level. With the increased loss of serum protein in the urine which followed the extraction of a tooth there seems to have been a slight decrease in its concentration in the blood, a value of 3.54 per cent obtained at the end of Period 67 being the lowest observed at any time during the patient's stay in hospital. By the end of the next group of basal periods there was again a slight rise which continued after his discharge. Serum obtained under basal conditions three months later contained 3.9 per cent protein.

Proteinuria increased whenever the protein of the diet was increased. Ingestion of egg white

caused a greater increase in proteinuria than any other protein except liver residue. However, in the case of the latter the average value given in the table is unduly weighted by the presence of marked proteinuria in Period 67. We believe that this should be attributed to temporarily increased permeability of the glomerular capillaries to protein caused by extraction of the tooth. If this surmise is correct, the protein of egg white should probably be regarded as more effective in the production of proteinuria and by inference in the formation of serum protein than the other proteins.

The increase of the albumin: globulin ratio of the urinary proteins during the egg white periods indicates that formation of albumin was especially rapid. This is in keeping with the observations of Pomerence, Slavin, Kariher and Whipple (20). The ratio and total amount excreted remained high during subsequent control periods. To account for this, one might infer that increased reserves of albumin were being discharged. Although there was a certain uniformity of the albumin: globulin ratio of the urine when entire periods or groups of periods were considered, values obtained on successive days on the same regime showed extremely great variation and were of doubtful clinical significance.

Case 2, P. B., Degenerative Bright's disease. The patient was an unusually well preserved man aged 59 years, a cabinet maker by trade, who had always had good health until the onset of the present illness. He entered the hospital on April 4, 1934 because of gradually increasing edema of the face, the lower portion of the trunk, the genitalia, and the lower extremities. His symptoms were of five weeks' duration. Insofar as could be ascertained there was no hematuria at the onset of his illness, nor any acute infection preceding it. From the history given, his dropsy had apparently become much worse after subsisting on a milk diet and drinking large quantities of water for the three weeks immediately prior to his admission. His blood pressure was 145/95 mm.

Hg. There was no evidence of cardiac failure. The face was slightly puffy; there was generalized edema of the subcutaneous tissues as high as the costal margin and moderate ascites. The teeth were poorly preserved; several were devitalized, and there was moderately severe pyorrhea. The specific gravity of the urine after restriction of fluid was 1.032. The excretion of urinary protein approximated 12 grams in 24 hours. The sediment obtained after centrifugalization contained many hyalin casts, a few granular casts and large numbers of epithelial cells and leukocytes. The sediment also contained a few cells resembling red blood cells, but these did not exceed 170,000 in twelve hours by Addis count. The standard urea clearance was 83 per cent of the normal

TABLE IV  
Case 2, P.B.—Protein metabolism

Period	Protein intake	Calories per diem	Weight	Protein metabolism				
				In-take	Catabolized protein	Urine protein	Deposited protein	Total protein synthesized
3 days each	grams per diem		kgm.	grams N per period	grams N per period	grams N per period	grams N per period	grams N per period
1.....	Diet 51	1520	69.7	24.61	34.62	4.33	-14.34	0
2.....			64.4					
3.....			61.3					
4.....	Basal diet 53	1930	59.8	24.61	29.1	3.58	- 8.07	0
5.....			59.7	24.61	25.1	2.91	- 3.40	0
6.....			59.8	24.61	25.1	2.91	- 3.40	0
7.....	Basal diet 53 Liver residue protein 50	2133	59.8	25.66	22.76	2.44	0.46	2.90
8.....			59.7	25.66	22.23	2.08	1.35	3.43
9.....			59.6	25.66	22.03	1.60	2.03	3.63
10.....			59.4	49.66	43.09	1.91	4.66	6.57
11.....	Basal diet 56 Liver residue protein 50	2814	59.4	49.66	42.00	2.27	5.39	7.66
12.....			59.1	49.66	42.21	2.36	5.09	7.45
13.....			58.8	49.66	43.02	2.20	4.44	6.64
14.....			60.2	51.19	38.97	2.50	9.72	12.22
15.....	Basal diet 53	1930	60.4	51.19	40.22	2.34	8.63	10.97
16.....			60.8	51.19	40.48	2.48	8.23	10.71
17.....			60.0	25.66	23.79	2.07	- 0.20	1.87
18.....			59.9	25.66	24.27	1.86	- 0.47	1.39
19.....	Basal diet 56 Serum protein 50	2814	59.4	25.66	22.68	1.48	1.5	2.98
20.....			59.5	25.66	23.48	1.23	0.95	2.18
21.....			59.5	51.19	39.50	1.51	10.18	11.69
22.....			59.7	51.19	38.74	1.85	10.60	12.45
23.....	Basal diet 56	2609	59.9	51.19	39.02	1.77	10.40	12.17
24.....			59.9	51.19	40.39	1.86	8.94	10.80
25.....			60.2	27.19	23.95	1.68	1.56	3.24
26.....			60.1	27.19	23.23	1.45	2.51	3.96
27.....	Basal diet 56 Liver residue protein 100	3019	60.1	27.19	21.70	1.31	4.18	5.49
28.....			60.2	75.19	48.44	1.50	25.25	26.75
29.....			60.0	75.19	56.08	1.88	17.23	19.11
30.....			60.4	75.19	59.19	1.94	14.06	16.00
31.....			60.4	75.19	59.14	1.97	14.08	16.05
32.....			60.0	75.19	55.77	1.79	17.63	19.42
33.....	Basal diet 56	2609	60.5	75.19	58.33	1.96	14.90	16.86
34.....			60.4	27.19	31.95	1.52	- 4.76	0
35.....	Basal diet 56	2609	60.2	27.19	26.72	1.30	- 0.83	0.47
36.....			60.2	27.19	26.72	1.30	- 0.83	0.47

average. The concentration of the serum protein was reduced, total protein 3.6 per cent with an A:G ratio of 1. The serum contained 652 mgm. per cent of fatty acid, 452 mgm. per cent of cholesterol and 122 mgm. per cent of lecithin. There was no anemia. The basal metabolic rate was 8.0 per cent below the normal average (Aub-DuBois).

*Diets.* During the first six days in the hospital his daily diet provided approximately 1800 calories, and contained 110 grams of protein, 1500 ml. of fluid, and a minimum of salt. There was practically no change in his condition during this time. On the seventh day (April 10, 1934) the protein of the food was increased to 150 grams; the caloric and fluid intake remained the same. On April 13th and 14th one and one-half grams of theocin were administered, and a marked diuresis followed. On April 20th he was transferred to the metabolism division for study.

During the first three periods he received a diet which furnished 1520 calories, contained 157 grams of carbohydrate, 72 grams of fat and protein (in grams) as follows: Vegetable, 11.5; milk, 15.1; egg, 7.1; meat, 16.3; Total, 50. Analyses showed that the food contained

24.61 grams of nitrogen per period or 51 grams of protein per day (see Table IV). Fluid intake was fixed at 2000 ml.

Beginning with Period 4, orange juice, grape juice, sucrose and cream were added in amounts sufficient to furnish 300 calories from carbohydrate and 110 calories from fat, leaving the protein content practically undisturbed. Actual analysis showed that there was an increase of two grams of protein daily from the added cream. This diet has been designated the *1930 calorie basal diet*. A supplement of 50 grams of liver residue protein was added to the above diet during Periods 7 to 10 (see Table IV and Figure 2).

During Periods 11 to 13 and 18 to 32 the caloric value of the diet was further increased, by addition of 440 calories from carbohydrate and 240 calories from fat. This has been termed the *2610 calorie basal diet*. Analysis for nitrogen showed an increase above the 1900 calorie diet equivalent to 3 grams of protein daily. Fifty grams of liver residue protein were superimposed on this diet in Periods 11 to 13, and 100 grams in Periods 25 to 30. Fifty grams of beef serum protein were added during Periods 18 to 21 (see Table IV and Figure 2).

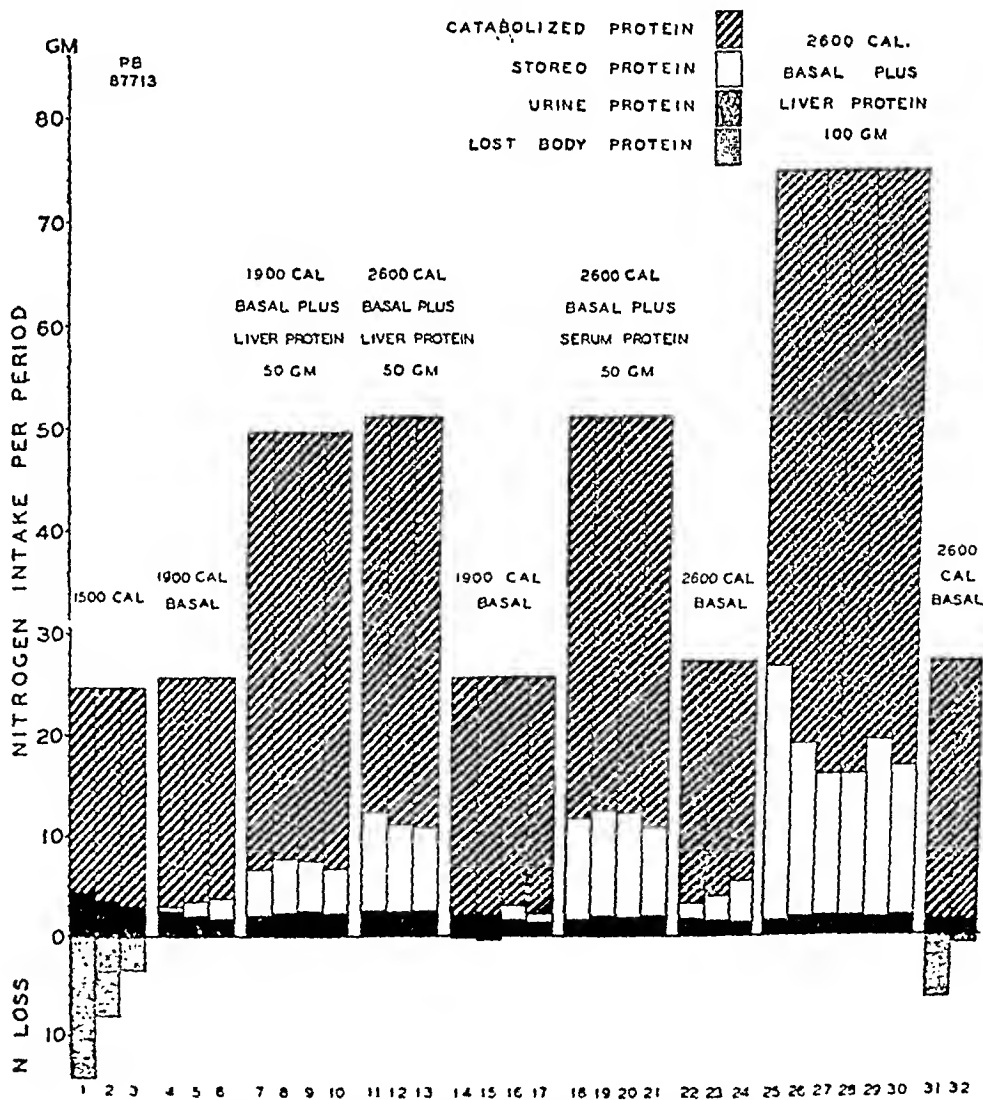


FIG. 2. CASE 2, P. B.—PROTEIN METABOLISM.

*Protein metabolism*

*Periods 1 to 3.* Diuresis and loss of edema which began prior to admission to the metabolism division continued during the first three periods. The total decrease in weight amounted to 23 kgm. of which 10 kgm. were lost in Periods 1 to 3. The proteinuria accompanying the diuresis was much greater than that of subsequent periods. In part, this may have been due to a residual effect of the previous high protein feeding, and in part to an increased rate of glomerular filtration. During these three periods in which the total calories given were 1500 per diem the apparent catab-

Figure 2 and Table V show that more protein was deposited with twice than with one and a half times the basal caloric requirement. The utilization of this protein with the higher caloric intake was practically the same as in the case of Patient Number 1 (compare Tables II and V).

The fact that the liver residue protein, when added to the 2600 calorie diet, was equally well utilized in quantities of 50 or 100 grams was entirely unexpected and similar to the utilization of lactalbumin by Patient L. R.

*Beef serum protein* was utilized with about the same efficiency by this patient as by the first, perhaps very slightly better. The amount stored did

TABLE V  
*Case 2, P.B. Utilization of dietary and supplementary proteins*

Description of diets	Calories per kgm. per day	Per cent of total calories furnished by protein	Average nitrogen of food	Average nitrogen of supplement	Average total nitrogen intake	Average nitrogen synthesized into protein	Per cent of intake synthesized into protein	Per cent of supplement synthesized into protein
		<i>per cent</i>	<i>grams per period</i>	<i>grams per period</i>	<i>grams per period</i>	<i>grams per period</i>	<i>per cent</i>	<i>per cent</i>
Basal, 1930 calories (5, 6, 16, 17)....	32	11	25.66		25.66	3.05	12	
Liver residue protein, 50 grams per diem (7 to 10).....	35.5	20	25.66	24.0	49.66	7.08	14	17
Basal, 2610 calories (23 to 24).....	43.5	9	27.19		27.19	4.72	17	
Liver residue protein, 50 grams per diem (11 to 13).....	47.0	15.5	27.19	24.0	51.19	11.30	22	27
Serum protein, 50 grams per diem (18 to 21).....	47.0	15.5	27.19	24.0	51.19	11.77	23	29
Liver residue protein, 100 grams per diem (25 to 30).....	50.0	20.5	27.19	48.0	75.19	17.49	23	27

olism of protein was considerably in excess of the intake.

*Basal periods.* The basal caloric requirement of this patient, as determined by oxygen consumption, was 22.5 calories per kilo daily. Body weight during Periods 5 and 6 was practically constant, and activity was very moderate. The 1930 calorie diet, which furnished 32 calories per kilo per diem was sufficient for dynamic purposes and also for deposition of protein. When the caloric intake of the basal diet was increased to 43.5 calories per kilo per diem by the addition of carbohydrate and fat, the utilization of protein was increased (Table V).

*Liver residue protein.* Just as with the basal diet, a better utilization of the same amount of this protein was obtained when more carbohydrate and fat were available for dynamic purposes.

not decline from period to period as it did in the first patient. This may indicate that the tissues of Patient Number 2 were more depleted at the time serum protein was fed.

*The nonprotein nitrogen of the blood, serum proteins and proteinuria (Table VI).* The non-protein nitrogen of the blood followed the expected course, rising with the increase in the intake of protein, and falling when protein was again restricted. Disappearance of edema was attended by a rise of about 0.5 per cent in the concentration of serum proteins, and another increase of 0.56 per cent occurred when 50 grams of liver protein were added to the basal diet. Increase in the A:G ratio accompanied the rise of serum protein. It is not at all certain that the higher protein intake (liver supplement of 50 grams) effected the second increase in serum protein. Dis-

TABLE VI  
Case 2, P.B.

Period	Diet	Blood non-protein nitrogen *	Serum proteins *		Total circulating serum protein *	Urine protein	
			Total	A : G ratio		Total	A : G ratio
<i>3 days each</i>		<i>mgm. per cent</i>	<i>per cent</i>		<i>grams</i>	<i>grams per period</i>	
1 to 3.....	1500 calories.....	31	3.56	1.2			
4 to 6.....	Basal 1900 calories.....	27	4.08	1.3	106	22.9	6.0
7 to 10.....	Basal 1900 calories+50 grams liver protein	18	4.20	1.4	107	12.9	8.6
11 to 13.....	Basal 2600 calories+50 grams liver protein	28	4.76	1.6	117	13.6	5.6
14 to 17.....	Basal 1900 calories	30	4.61	1.6	124	15.4	7.0
18 to 21.....	Basal 2600 calories+50 grams serum protein	20	4.68	1.7	131	10.4	4.8
22 to 24.....	Basal 2600 calories	23	4.75	1.97	124	10.8	4.2
25 to 30.....	Basal 2600 calories+100 grams liver protein	19	4.72	1.7		9.1	4.7
31, 32.....	Basal 2600 calories	30	4.64	2.1		11.5	8.1
33, 34.....	Mixed high protein 3000 calories	25	4.92	1.8		8.8	3.0
3 months after discharge.....		27	5.08	1.7		10.2	3.6
6 months after discharge.....		33	5.4	1.8			
		28	5.8	2.2			

\* Blood nonprotein nitrogen and serum protein determinations were made at end of each group of periods.

tinct clinical improvement was noted before the feeding of liver protein was started, and inspection of successive series of basal periods shows a tendency for less and less protein to be lost in the urine. Decrease in proteinuria combined with adequate caloric intake and a reasonably abundant supply of protein of good quality was probably sufficient to permit a rise in the level of serum protein.

After the heavy loss of protein in the urine during the initial diuresis, the addition of extra protein to the diet was accompanied by increased proteinuria over proximate control periods (Table VI). This tendency was somewhat indistinct owing to the gradual decrease of proteinuria during the control periods, and was more marked in the previous patient (L. R.). The A:G ratios of the urinary proteins varied greatly and are of doubtful significance.

*Case Number 3.* Degenerative stage of chronic hemorrhagic Bright's disease. R. P. was a 40 year old janitor who gave a history of having had hematuria 20 years before admission and a cystitis of many years' duration. During the two weeks before admission he had developed edema of the ankles and legs and had gained ten pounds in weight. For one week he had had pain in the epi-

gastrium, which gradually became more severe. Physical examination on admission revealed a subcutaneous mass in the epigastrium which was tender and not reducible. The blood pressure was 140/80. The heart was slightly enlarged to the left and there was edema of the legs. The urine contained a large amount of albumin, and the sediment contained red blood cells, epithelial and white blood cells, and many casts. The blood contained 14 grams of hemoglobin per 100 cc., 4.5 million red cells and 6300 leukocytes per cubic millimeter, and 50 mgm. per cent of nonprotein nitrogen.

The epigastric hernia was repaired under local and gas-oxygen anesthesia. After the operation, the urinary protein was found to range from 15 to 20 grams daily, and seven days after operation the total serum proteins were 4.05 per cent. He was placed on a high protein diet but because of nausea was unable to take the prescribed amount of food for about 10 days. On August 28th, twenty-one days after operation, his serum proteins were 3.1 per cent. The standard urea clearance was 63 per cent on September 2nd and 60 per cent on December 4th.

*Diets.* Beginning September 1st he was given a salt-free diet which furnished 3200 calories and contained 100 to 120 grams of protein. Period 1 in Table VII represents the last three days of this diet. During Periods 2 to 20 he received a basal diet which contained 60 grams of protein, and had an energy value of 3200 calories. Fifty grams of liver residue protein per diem were added to the diet during Periods 15 to 17 and 100 grams of the same protein during Periods 18 to 20. Fluid intake was fixed at 2000 ml.

TABLE VII  
Case 3, R.P.

Date or period	Daily diet	Serum proteins		Urine protein		Blood non-protein nitrogen	Approximate nitrogen intake	Total urine nitrogen	Body weight	Edema
		Total	A : G ratio	Total	A : G ratio					
1934		per cent		grams per diem		mgm. per cent	grams per diem	grams per diem	kilos	
August 11...	As tolerated	4.05	0.5			44				+++
August 28...		3.06	1.0			38				+++
September 1.	Calories 3200 Protein 100 to 120 grams Salt free	3.14	1.0	13-18		34	16-20	8-10	68.8	++++
September 18.....									57.8	0
October 15...		3.17	1.0			33			60.5	0
October 27...		3.10				38			65.0	0
Period 3 days each				grams per period			grams per period	grams per period		
1.....		3.1		56.8	4.0	35	50	27.9	65.4	0
2 to 5...	Basal diet Calories 3200 Protein 60 Salt free	2.95	1.9	44.3	4.0	29	28.0	19.8	64.2	0
6.....				*	*		28.0	*	64.8	0
7.....				42.2	4.7		28.0	22.6	65.0	±
8 to 10...				*	*		28.0	*	65.6	±
11.....				42.9	6.0		28.0	20.1	65.4	±
12, 13				*	*		28.0	*	64.8	+
14		2.9	1.3	40.5	4.7	32	28.0	22.2	64.6	+
15 to 17..	Basal plus liver residue protein 50 grams	3.0	1.4	56.4	5.5	36	52.8	34.2	63.8	0
18 to 20..	Basal plus liver residue protein 100 grams	3.0	1.6	68.4	5.7	45	76.8	50.1	64.4	0

\* Figures for these periods not available.

*Protein metabolism*

The decrease in serum proteins following the operation has already been mentioned. Several factors may have been responsible for this, the most obvious of which was the inability to take an adequate diet. The operative procedure and the anesthetic may have been responsible either for additional capillary damage and consequent increased renal permeability with loss of more protein in the urine, or a decrease in the ability to manufacture serum proteins.

With the ingestion of an adequate diet (September 1st) he was able to store protein. The exact amount deposited is not known, but the difference between the nitrogen intake and urinary output in the presence of a stationary blood nonprotein nitrogen was sufficient to indicate a positive protein balance. The loss of 11 kgm. in weight after September 1st was associated with loss of edema. Subsequent gain in weight was not associated with evidence of edema, giving further proof that pro-

tein was stored. In spite of this there was no rise in serum proteins.

The diet of Periods 2 to 14 inclusive contained 60 grams of protein, which, in view of the heavy proteinuria, was intended to be insufficient to maintain the concentration of serum protein at its previous level. The object of giving the lower amount of protein was to deplete the body's store of plasma protein, but the nitrogen balance observed in these periods did not point to a loss of body protein. The protein-sparing action of the high caloric diet (3200 calories) evidently permitted efficient utilization of the food protein and maintenance of nitrogen equilibrium. Proteinuria fell considerably below the previous level, and there was a very slight decrease in the concentration of serum protein. This was accompanied by slight but definite edema.

When the protein intake was increased by the addition of "liver residue" protein to the diet in Period 15, nitrogen was deposited and there was



a marked increase in proteinuria. Edema disappeared after 3 or 4 days but the serum proteins failed to rise above 3 per cent.

#### DISCUSSION

*The relation of tissue protein to serum protein.* The filling of tissue reservoirs with protein did not influence the quantity of circulating serum protein in the nephritic patient. During seven months, Case Number 1 retained nitrogen equivalent to 2.8 kilograms of protein, yet there was no increase in the level of serum proteins, and at a given level of protein intake, proteinuria was no greater at the end than at the beginning of the experiment. The total amount of protein which can thus be deposited in the body is not known. Rubner and other investigators (3, 6, 14) have believed it to be relatively large. Deuel et al. (6) noted the loss of 1.6 kilo of protein while subsisting for 81 days on a nearly protein-free diet. Part of the deposit appeared to be in the form of a labile fraction which was readily broken down when the intake of protein fell below an optimum level. Further evidence that deposit protein may consist of more than one fraction has been given by Pomeroy, Slavin, Kariher and Whipple (20). Their observations on the regeneration of plasma proteins in the dog indicates the necessity of drawing a distinction between the total reserve or deposit protein and that fraction of it which is readily available for conversion into plasma protein. Using the data which they have given one may estimate that an average sized man might have as much as 500 grams of serum protein held in special reservoirs. It is to be recognized, of course, that species differences may invalidate such a computation. The observations made on the nephritic patient give us no definite evidence that any of the retained protein was used to build up a reserve to protect the body against further depletion of serum proteins. On the other hand, there is no proof that all or part of the reserve set up in Case 1 could not have been used to help maintain the existing level of the serum proteins.

*The ability of nephritic patients to synthesize serum protein.* In a discussion of the problem of hypoproteinemia Bloomfield (2) has cited arguments in favor of an impaired or injured mechanism for regeneration of serum protein in the

nephritic. On the basis of available information Weech, Goettsch and Reeves (24) are inclined to agree with Bloomfield that the loss and lack theory is insufficient to account for hypoproteinemia, but they consider it hardly justifiable to assume that there is an injury to the blood protein-forming mechanism. Although from the observations at hand one can not tell whether any of the protein being deposited in the tissues of Patients 1 and 2 served as a defense against further depletion of the serum proteins, it is possible by measurement of proteinuria to produce evidence of a very active regenerative process in Patients 1 and 3. To refer again to Case 1, it will be noted that during 213 days the patient was able to produce 5.5 kilograms of protein, beside meeting the ordinary requirements of his protein metabolism. Of this amount 2.8 kilograms were held in the tissues, and 2.7 kilos escaped in the urine. Since a considerable degree of undersaturation of the tissues with protein is indicated in the initial periods, it is probable that any reserve stores of serum protein had been depleted before these studies were begun. As a consequence the regenerated serum protein appears to have come from the protein of the food. During the stated interval of time the construction of new serum protein, therefore, amounted to about thirteen times the total quantity of circulating serum protein in a normal individual of similar weight. As the patient's total circulating serum protein was only about half as great as that of the normal, regeneration in his case was sufficiently rapid to have replaced all of his serum protein within ten days. Likewise Case 3, who had marked hypoproteinemia, was able to regenerate about 14 grams of serum protein per diem. The diet in the latter instance contained only 60 grams of mixed protein daily, and after the requirements of metabolism and proteinuria had been met there was little protein available for deposition in the tissues. At no time was there a negative nitrogen balance, and no appreciable decrease in the concentration of serum protein occurred. The catabolism of protein was 0.72 gram per kilo per day showing an efficient utilization of food protein.

The hypothesis of defective regeneration of serum proteins in nephrotic states seems to have been substantiated to a certain extent in Case 2.

The diet appears to have furnished the calories and protein required for effective replacement of tissue protein, and the losses of protein in the urine gradually became much less than in Cases 1 and 3, yet the increase in circulating serum protein was exceedingly slow. Before ascribing the small increase in circulating protein to faulty regeneration, a number of other possibilities must be considered. As Whipple and his collaborators have shown (10, 20), a reserve depot of serum protein or readily available precursors exists in the body. It is hardly possible to conceive of replacement of serum protein without some replacement of parent material. That such deposition occurred is suggested by delayed increase in proteinuria following addition of supplementary protein to the diet and by excess excretion of urinary protein for a varying number of days after the supplement was discontinued. A similar lag has been noted in the dog (10).

Little as yet is known of the mechanisms which stimulate the formation of serum protein. Evidently artificial lowering of the concentration by removal of the protein is an effective stimulus to the regenerative process in the normal dog (10). The increased catabolism of protein incident to starvation in one of Weech's dogs (24) suffering from nutritional hypoproteinemia seems to have provided the requisite stimulus for regeneration of serum protein. This result is diametrically opposed to the result obtained in a somewhat similar experiment reported by Pomeranke, Slavin, Kariher and Whipple (20). In the latter instance, the reserves of plasma protein had been depleted prior to fasting, and only minimal formation of new plasma protein occurred. One might suspect, therefore, that in the case of Weech's dog, reserves of the precursors of serum protein still existed, and that the utilization of body protein for dynamic purposes in some way liberated them to replace losses from the blood.

Clinical observations have shown that the proteinuria accompanying a concentration of serum protein of 3 per cent is generally much greater than that accompanying a concentration of 5 per cent (9). One might conjecture that one of the reasons that a particular degree of hypoproteinemia is maintained over a considerable period of time is because the stimulus to regeneration is just

sufficient to offset the losses in the urine. Until the rate of escape of protein decreases either as a result of improvement in the renal lesion or because the number of functioning glomeruli has been greatly reduced (renal failure) little change in the circulating serum protein is to be expected. Nevertheless, it is difficult to reconcile this view with other known facts. It has already been shown that different kinds of food protein cause different rates of regeneration in the dog (10, 20). The evidence submitted here appears to point quite definitely to an increased rate of formation of serum protein in the periods in which the intake of protein was increased, but with respect to the animal proteins tested, quantity seems to have been fully as important a factor as quality in increasing proteinuria. If the production of serum protein can be increased by the means indicated, it is remarkable that an increased rate of manufacture should have been completely offset by the increased rate of escape of protein in the urine. It is extremely doubtful whether the greater proteinuria can be attributed to further injury to the kidney. Tests of renal function as well as general improvement in the clinical condition of these and other patients (8, 13) do not point to increased renal damage.

Since the albumin fraction appears to have been relatively more depleted than the globulin, it might be supposed that high protein diet would lead to a greater formation of albumin than globulin and hence to a greater escape of the smaller albumin molecule in the urine. Were this true, the urinary ratio of albumin to globulin should have risen in high protein periods. Actually, variations in the ratio were so inconsistent that they failed to lend any support to this hypothesis, some of the highest ratios being observed when the protein intake and proteinuria were the least. The diuretic effect of high protein diets has been shown to cause increased renal blood flow (22) and is probably a real factor in increasing the output of protein in the urine. Some preliminary experiments with diuretin and urea in Case 3 showed that proteinuria definitely increased when these substances were administered. While it does not seem feasible at present to attribute the hypoproteinemia of nephritis to any single influence, the amount of protein lost in the urine seems to have had an im-

portant effect on the degree of hypoproteinemia. The two patients (Cases 1 and 3) who showed a high grade of proteinuria had persistently low concentrations of serum protein and were unable to increase the amount of blood protein in circulation. The increase in serum protein in Case 2 was apparently closely related to decreasing losses of protein in the urine. Again there was no obvious relationship between high protein intakes and the level of the serum proteins.

*Lag in excretion of nitrogen and protein* requires brief mention. Falta and others (7, 4, 14) have found that in normal individuals considerable lag in the excretion of nitrogen may follow the superposition of certain proteins on a standard diet. Lag has been attributed to the manufacture of deposit protein and its subsequent utilization in metabolism when the supplementary protein feeding was stopped. Of the proteins tested, egg white was found to produce the longest lag. This is of interest in connection with the ingestion of cooked egg white by Case 1. The greatest delay in return of proteinuria to the basal level occurred after feeding this protein.

*The efficiency of utilization of supplementary protein* at different levels of intake varied considerably. It has already been mentioned that increasing increments of egg white and serum protein were less well utilized, while the efficiency of utilization of 50 and 100 gram supplements of either lactalbumin or liver protein remained constant. The lack of uniformity in these results suggests, that, providing the energy furnished by carbohydrate and fat is sufficient, the utilization of dietary proteins depends upon the nature of the protein required by the body and the quantity and relative proportions of the amino acids furnished by the metabolic mixture.

Mitchell (15, 16) found that there was a decrease in the biological value of protein for growing rats when the concentration in the diet was increased from 5 to 10 per cent. He attributed this decrease partly to less effective utilization for growth than for maintenance, the maintenance requirements being met first and such of the available amino acids as were left then being employed to form body protein. In addition, when higher concentrations of protein were fed, the concentration of amino acids in the nutrient mixture sur-

rounding the cells was thought to have increased, and thus led to greater utilization of protein for dynamic purposes.

The data of von Hoesslin (23) on malnourished individuals are more comparable to the results obtained in nephritis than are animal experiments. He found in most instances that increasing increments of food protein were less effectively utilized but cites at least one example where the percentage of nitrogen retained when 187 grams of protein were fed daily was greater than the percentage retained when only 97 grams were fed. Each diet provided nearly the same number of calories in the form of carbohydrate and fat.

As Mitchell has suggested, one can hardly expect the biological value of a protein to be the same for all functions and the phenomena observed in our patients may not be true of other conditions. The ability to utilize some proteins more effectively than others may have depended upon the large demand for a mixture of amino acids suited to the formation of plasma protein and its precursors. Those proteins, then, which were most effectively utilized provided in addition to the amino acids needed for regeneration of plasma protein a suitable mixture of building stones for formation of deposit protein.

#### SUMMARY

The protein balances of three patients with degenerative Bright's disease, heavy proteinuria and hypoproteinemia have been measured. The method of procedure was to superimpose on basal diets different proteins or additional calories in the form of carbohydrate and fat.

When the caloric and protein contents of the diet were sufficient, all of the patients stored large amounts of protein, an indication of previous depletion of their tissue proteins.

In the case of Patient 2, when the intake of protein approximated 0.8 gram per kilo daily, a diet with a caloric content of one and one-half times the basal requirement permitted some storage of protein. Further additions of carbohydrate and fat sufficient to raise the caloric content of the diet to twice the basal requirement appreciably increased the deposition of protein.

The sum of the protein stored plus protein lost

in the urine was taken as the total amount of protein synthesized in the body from food. The percentage utilization of the proteins of the food was computed on this basis (Tables II and V).

Small supplementary feedings of egg white and serum protein (supplements of 10 to 25 grams) were more efficiently utilized than supplements of 50 grams. Lactalbumin and the protein of liver residue were utilized equally well when either 50 or 100 grams were fed daily.

When the protein intake was increased, proteinuria increased. This has been considered as evidence pointing to an increased rate of synthesis of serum protein. The escape of a larger amount of protein in the urine may have been due to the increased rate of blood flow through the kidneys resulting from the diuretic action of the high protein diet.

While ingesting a high protein diet, two of the patients failed to show any increase in the total amount of circulating serum protein although they were able to deposit large amounts of protein in the body. In both instances the intensity of the renal disease, as indicated by the character and amount of formed elements in the urinary sediment, remained stationary.

The concentration of the serum proteins of the third patient (Case 2) increased apparently because the renal lesion improved.

Experimental evidence has been adduced to show:

(1) That the ingestion of supplementary protein leads to an increase in proteinuria, but only after a latent period of several days.

(2) That the increased proteinuria persists for a few days after the high protein diet has been discontinued.

These facts suggest a partial equilibrium between serum protein and that part of the deposit protein which serves as a reservoir of serum protein.

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# THE ADDIS SEDIMENT COUNT AND BLOOD UREA CLEARANCE TEST IN NORMAL PREGNANT WOMEN

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Many tests have been done to evaluate renal function in the toxemias of pregnancy and investigators (1, 2, 3, 4, 5) are agreed that the most accurate is the blood urea clearance. Addis (6, 7) has studied the urinary sediment in both normal and nephritic patients and is able by this means to classify the nephritides more accurately. These two tests are the most sensitive and accurate of any used to determine the character and extent of damage of the kidneys. However, there is a scarcity of Addis count values in normal pregnant women. Both tests have been used in this clinic to find the limits of variation in normal women in the last trimester of pregnancy, as a means of obtaining a better basis for comparison of values obtained in cases of toxemia, which have their onset during this period.

## METHOD

On the first day the patient was admitted to the hospital after the usual breakfast including tea, coffee or milk. The patient was instructed not to drink more fluids or eat more fruit than usual for breakfast. The patient remained in bed until the completion of all tests and no fluids, "ANYTHING THAT CAN BE POURED," were given for twenty-four hours. The regular house diet exclusive of tea, coffee, milk or ice cream was given, but no more fruit than usual even if the patient complained of thirst. The bladder was emptied at 7.30 p.m. and the urine discarded. The patient was encouraged not to void after this time.

On the second day the following routine was carried out: breakfast was delayed until the completion of all tests. The patient was catheterized at 7.30 a.m. and the catheter retained in the bladder until the completion of all tests. The specimen of urine obtained at 7.30 was put in a bottle specially prepared for the Addis count. During the next two hours one liter of water was given to

insure obtaining a sufficient volume of urine for measuring the urea clearance. At 9.30 a.m. the bladder was completely emptied, the urine discarded, and the patient was given 500 cc. more water. At 10.30 a.m. and again at 11.30 a.m. the bladder was completely emptied, both specimens of urine saved and the catheter removed. After the 10.30 catheterization a venipuncture was done and the patient was given 500 cc. more water. It is extremely important that the bladder be completely emptied on time and that the patient drink all of the water designated.

## LABORATORY PROCEDURE

The urinary sediment count was done according to the method of Addis (6, 7). The urea of the blood and urine was determined by Van Slyke's method (8), and the blood urea clearance was calculated (9). The total proteins of the urine were determined in the supernatant urine in the Addis tube, according to the method of Shevky and Stafford (10).

## RESULTS

In the accompanying table are the observations made of nineteen normal women in the last trimester of pregnancy, three of whom were multiparas and sixteen primiparas. The tests were done from 1 to 83 days antepartum. The blood pressures of these women were all within normal limits. The average values of the urea clearances for the first and second hours varied from 60 to 118 per cent of the average normal as established by Möller, McIntosh and Van Slyke (9), six being below 80 per cent. The total protein content of the urine was within normal limits. The cast count varied from 0 to 10,000, except one case which showed a count of 14,000. This patient had had acute pyelitis one year previously, a fact which may account for the high value. The red blood cell count ranged from 47,000 to 1,900,000

TABLE I  
*The Addis urinary sediment count and blood urea clearance of normal pregnant women*

Case number	Age	Para	Days ante-partum	Blood pressure	Urea clearance		Protein-uria	Addis count		
					1st hour	2nd hour		Total casts	R.B.C.	W.B.C.
	<i>years</i>			<i>mm. Hg</i>	<i>per cent normal</i>	<i>per cent normal</i>	<i>grams</i>		<i>thousands</i>	<i>millions</i>
1	18	0	33	120/70	64.3	79.6	.36	7,500	1,192	2.30
2	18	0	31	105/60	68.2	68.0	.25	0	285	0.60
3	35	V	55	120/60	87.7	105.0	.36	14,000**	500	0.50
4	20	0	32	122/80	70.0	90.0	.18	0	367	3.18*
5	21	0	18	130/70	69.0	102.0	.36	7,400	22,230	6.00*
6	20	0	60	120/80	122.8	96.8	.18	9,900	Trauma 8,300	2.30*
7	17	0	25	115/70	85.5	78.3	.20	0	1,943	0.11
8	22	0	30†	130/90	120.0	105.0	.36	0	510	2.00*
9	32	0	42	120/62	51.2	86.8	Trace	0	1,000	3.15
10	20	0	24	120/60	85.9	75.0	Trace	0	670	0.50
11	24	0	30	118/74	68.0	68.0	.36	10,000	570	0.62
12	18	0	7	90/60	85.9	74.6	.36	9,702	525	0.53
13	23	0	42	100/50	62.5	57.9	.40	0	440	0.55
14	26	0	3	116/60	90.4	80.0	.36	0	254	0.27
15	18	0	16	118/80	88.4	49.2	.72	0	4,810	12.90*
16	25	II	1	110/50	119.0	103.0	.30	1,000	Trauma 102	0.03
17	24	III	3	130/60	132.0	105.0	0	8,800	310	0.62
18			1		Not done—labor began		.36	0	342	2.00
19	22	0	83	100/60	78.0	84.0	.03	3,200	47	0.10

\* Bacteriuria.  
† 21 days following test, developed toxemia.  
\*\* Acute pyelitis 1 year ago.

except in those cases in which there was known to be definite trauma in catheterization. The white blood cell count ranged from 25,000 to 6,000,000. In several instances there was a definite bacteriuria.

DISCUSSION

The values of the urea clearance established by Möller, McIntosh and Van Slyke (9) for non-pregnant normal subjects ranged from 80 to 120 per cent of the average normal clearance. We have found that the values ranged from 60 to 118 per cent of the average normal clearance in normal women in the last trimester of pregnancy, with one-third between 60 and 80 per cent. Therefore 60 per cent is probably the low limit of normal.

Addis found the urinary sediment of normal non-pregnant individuals to contain: casts 0 to 5,000; red blood cells 0 to 425,000; and white blood cells 32,000 to 1,000,000. Our observations showed casts varying from 0 to 10,000, with half of the cases having a count greater than 5,000, which is above the upper normal limit observed

by Addis. The red blood cell count varied from 47,000 to 1,900,000 except in three cases where there was definite trauma by the catheter. Among the remaining 16 cases, 5 showed an erythrocyte count greater than 500,000. The cell count of leukocytes and epithelium ranged from 25,000 to 6,000,000 with 8 instances in which it was greater than 1,000,000.

The wider limits of variation may be explained by the changes in the physiology and anatomy of women in the last trimester of pregnancy. Recently Coutts et al. (11) have demonstrated by aortograms of 12 women in the last trimester of pregnancy that the aorta is displaced to the left, that the renal arteries course upward and that the circulation in the common iliac arteries is altered. These facts suggest that pressure of the gravid uterus may alter the renal circulation. We have noted that patients after dehydration and catheterization usually secrete very little urine for the next 2 hours. This is in accordance with the findings of Dieckmann (5) who has called attention to the small volume of urine obtained antepartum with the larger volume obtained postpartum. The

ureters may become dilated by the pressure of the gravid uterus, thus giving rise to stasis, and possibly to bacteriuria and to an elevated leukocyte count in the urinary sediment. In view of these changes, it seems reasonable to suppose that renal function may be secondarily altered, thus accounting for the lower values of the urea clearance and the larger number of formed elements in the urinary sediment of normal women in the last trimester of pregnancy, when compared with the values previously obtained by others in non-pregnant normal individuals.

#### CONCLUSIONS

In nineteen normal women during the last trimester of pregnancy, counts were made of the number of formed elements in the urinary sediment, and measurements made of the urea clearance.

1. In the urinary sediment, using the technique of Addis, casts varied from 0 to 10,000; the red blood cells from 47,000 to 1,900,000; and the cell count of leukocytes and epithelium from 25,000 to 6,000,000. These values are higher than the corresponding values hitherto observed in normal non-pregnant individuals.

2. The values of the urea clearance varied from 60 to 118 per cent of the average normal standard established by Van Slyke and coworkers. From this it is apparent that the lower limit of normal urea clearance is somewhat less than in non-pregnant individuals.

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# INTUBATION STUDIES OF THE HUMAN SMALL INTESTINE. IV. CHEMICAL CHARACTERISTICS OF THE INTESTINAL CON- TENTS IN THE FASTING STATE AND AS INFLUENCED BY THE ADMINISTRATION OF ACIDS, OF ALKA- LIES AND OF WATER<sup>1</sup>

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WITH THE TECHNICAL ASSISTANCE OF

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The development of a practical technique by Miller and Abbott (1) for the prompt collection of jejunal and ileal contents from the normal human subject, using a double-lumened apparatus so arranged as to obstruct passage beyond the point of aspiration, has rendered possible for the first time a direct investigation of the chemical nature of these intestinal fluids comparable to the studies previously made on gastric and duodenal juices. The method permits, in addition, a more complete collection of duodenal contents than can be obtained with a single-lumened tube. Thus an opportunity is now available to make observations on material obtained in a satisfactory manner from any part of the small intestine of man.

In this presentation we report the results of chemical determinations made on contents from the duodenum, the jejunum and the ileum of individuals with apparently normal digestive tracts. They show the range in reaction, bicarbonate concentration and osmotic pressure, under fasting conditions and after the oral administration of hydrochloric acid, of sodium bicarbonate and of water. Some data on the chloride, calcium, phosphorus and total nitrogen concentrations are included. An attempt has been made to correlate the various observations. The results will be used as a basis of comparison for work now in progress in this Clinic on problems of intestinal secretion and absorption.

## METHODS

1. *Subjects.* The observations are based on fifty-one intestinal intubations carried out on thirty individuals,

<sup>1</sup> Aided by a donation from Mr. Samuel S. Fels and by grants from the Faculty Research Committee and from Smith, Kline and French Laboratories.

some of whom were patients whose gastro-intestinal tracts were considered to be normal and some, paid healthy subjects. Each subject took the tube in the morning after a 14 to 16 hour fast. The specimens included samples obtained consecutively at one intubation as well as samples from repeated intubations of the same individual.

2. *Intubation.* The method (1, 2) involved the use of a double-lumened tube with a thin rubber balloon attached over the distal end of one lumen. This balloon, when distended in the duodenum by the injection of air, stimulates peristalsis sufficiently to sweep the apparatus rapidly along the intestine, and then, when in the desired position and somewhat less tense, serves as a dam to facilitate aspiration of the contents above it through fenestrations in the second lumen. The efficiency of the balloon in checking the flow of contents was clearly established by roentgen examination after the oral introduction of barium sulphate suspensions. The specimens were aspirated by a negative pressure of 70 cm. of water, which is sufficient to conduct through a single lumen of a nine foot tube 150 cc. of intestinal juice per minute. As the end of the tube frequently reached the terminal ileum in the course of three hours after traversing the pylorus, the entire length of the small intestine was available for study.

Although both measurement of the length of the tube beyond the pylorus and fluoroscopy were employed, the exact point in the small intestine from which material was being aspirated was difficult to determine, except when near the cecum. Even when the tube was checked at the mouth and no more of it allowed to enter we observed that nevertheless more and more of the intestine tended slowly to assume a position above the balloon, thus causing the tube from time to time to reach an actually deeper level in the bowel. On one occasion, for instance, a balloon on a tube extending only four and a half feet beyond the pylorus lay in the upper ileum within three hours after it was swallowed, but after six hours, without any more tube being allowed to enter, it was found to be in the cecum. For this reason, and because we have found no abrupt change in the character of the contents or in the roentgen picture in differ-

taking place, collections of gas appeared interspersed between columns of fluid. The material in its gross appearance conformed, with slight variation, to the description of Miller and Abbott (2), being a somewhat viscid fluid, of amber to greenish color, and it usually contained fine flecks of mucus and other debris which settled on standing.

(b) *Reaction.* The pH was very variable, ranging from 2.64 to 7.80. There was a tendency for the contents to become more alkaline lower in the intestine, though distinctly acid specimens (pH 4.8) were occasionally recovered even from the ileum.

(c) As would be anticipated from the wide range of pH, the bicarbonate concentration was variable, our extremes being 1.3 and 45.5 m.eq. per liter.

(d) *Osmotic pressure.* The contents, especially from the upper intestinal tract, were often

hypotonic as compared with blood plasma. In their passage down the tract, however, isotonicity was usually attained, but never exceeded. The chloride concentration was in reciprocal relationship with the bicarbonate content, thus maintaining the osmotic pressure. The chloride ranged from 50.6 to 150.6 m.eq. per liter (296 to 880 mgm. NaCl per 100 cc.).

(e) *Calcium, phosphorus and nitrogen.* The calcium concentration was usually below the normal blood level. A few analyses indicated that practically all of the phosphorus was in the inorganic form, its concentration was usually above that of blood plasma. Both calcium and phosphorus tended to be more concentrated in the upper intestine. As the few analyses indicated that about one-half of the total nitrogen was precipitable by zinc hydroxide, the fasting protein content was very low.

## 2. After hydrochloric acid and sodium bicarbonate administration

When hydrochloric acid solution (0.16 N), isotonic with blood plasma, was introduced into the stomach by tube in 50 cc. quantities the resultant influence on the chemistry of the intestinal contents at various levels is shown in Figure 2. Hydrochloric acid of this concentration is apparently held in the stomach, and released into the intestine in small quantities so that neutralization is rapidly attained. No immediate increase in rate of flow was observed, but in two cases an increase occurred about one hour after giving the acid. No decrease in the pH but rather a tendency to an increase was noted, showing that the alkaline intestinal fluids are present in excess. Isotonicity with the blood plasma was maintained.

The response to isotonic sodium bicarbonate solution was in marked contrast to that which followed the administration of isotonic hydrochloric acid (Figure 3). The bicarbonate left the stomach very rapidly as is shown by the immediate increase in the rate of flow, in the bicarbonate content and in the alkalinity of the intestinal contents. Sodium bicarbonate in 5 per cent solution also left the stomach relatively rapidly. That it was not held until isotonicity was established is indicated by the osmolar concentration of the intestinal fluid rising appreciably above 300 milliosmoles. When

TABLE III

Concentrations of calcium, nitrogen and phosphorus in the intestinal contents of fasting subjects at various levels

Distance beyond the pylorus	Calcium	Total phosphorus	Total nitrogen
cm.	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.
Duodenum...	12.4		
Duodenum...		5.5	61
Duodenum...		4.7	33
30.....			63
30.....	8.0		
30.....	5.2	10.0	56
30.....	5.6	13.2	
30.....	11.6	7.1	
30.....			45
60.....	12.8	7.2	45
60.....	5.4	7.3	73
60.....	8.0		
60.....			55
60.....	5.8	7.1	50
90.....			109 *
90.....	5.4		
90.....	5.2	5.9	54
90.....			40
90.....		7.1	
90.....		4.5	
120.....	12.8		200 *
120.....	5.0	6.3	53
120.....	5.8	7.3	
120.....		4.5	
120.....		4.7	86
150.....	5.0	6.3	53
150.....	9.8		34
150.....			45
Average.....	7.7	6.8	53

\* Not averaged.

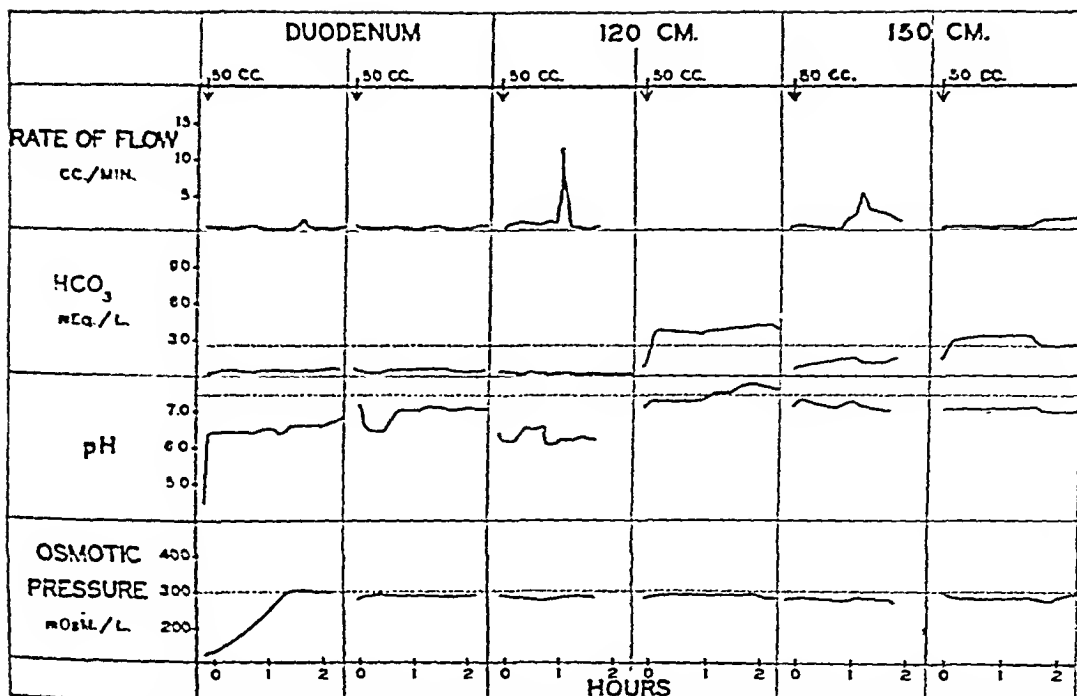


FIG. 2. CURVES TO SHOW THE EFFECTS OF THE ORAL ADMINISTRATION OF ISOTONIC HYDROCHLORIC ACID SOLUTION (0.16 N) ON THE CONTENTS OF THE DUODENUM AND OF THE ILEUM (120 AND 150 CM. BELOW THE PYLORUS).

Dotted lines indicate normal values for blood plasma. Arrows indicate time of administration.

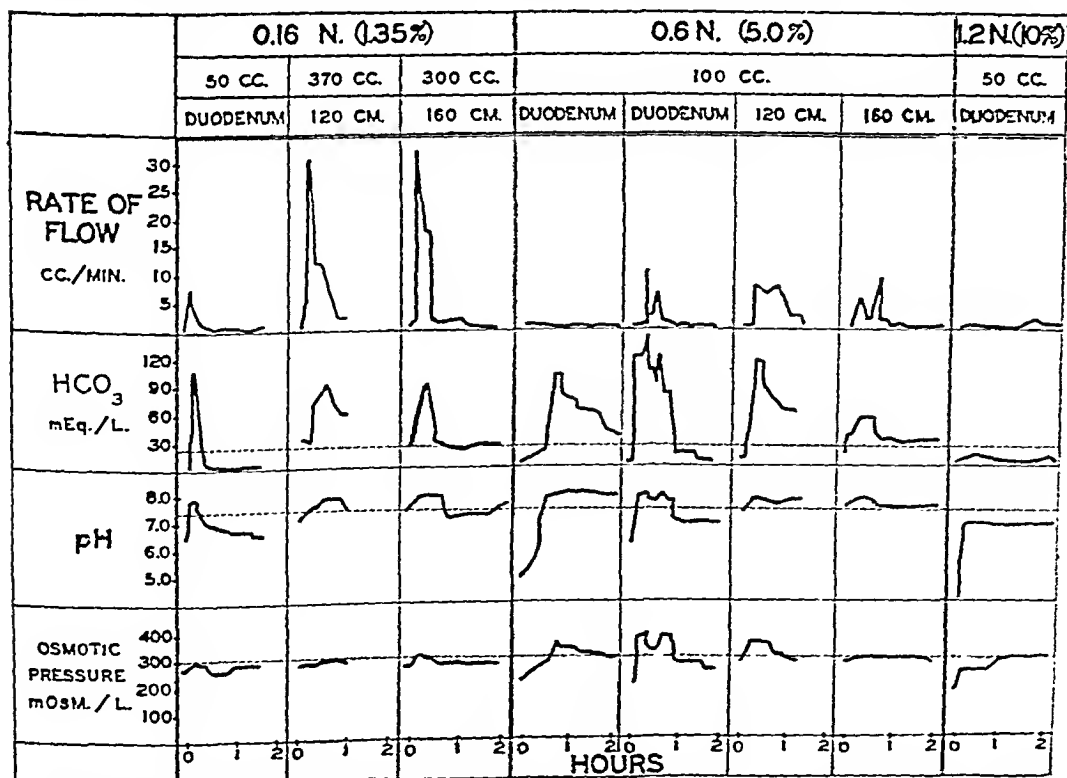


FIG. 3. CURVES TO SHOW THE EFFECTS OF THE ORAL ADMINISTRATION OF ISOTONIC AND HYPERTONIC SOLUTIONS OF SODIUM BICARBONATE ON THE CONTENTS OF THE INTESTINAL TRACT

Dotted lines indicate normal values for blood plasma. Solutions were administered at zero time.

in a single experiment a stronger solution of sodium bicarbonate (10 per cent) was given it was retained by the stomach until diluted. This is definitely indicated by the absence of an appreciable increase in the bicarbonate ion of the duodenal fluid. In the latter instance, furthermore, bicarbonate was present in the stomach three and one-half hours after its administration, in contrast to some experiments with isotonic bicarbonate solution in which the gastric juice again became acid within thirty minutes.

3. After water

The data obtained on specimens from three different levels in the intestine after the subject had drunk 400 cc. of water are plotted in Figure 4.

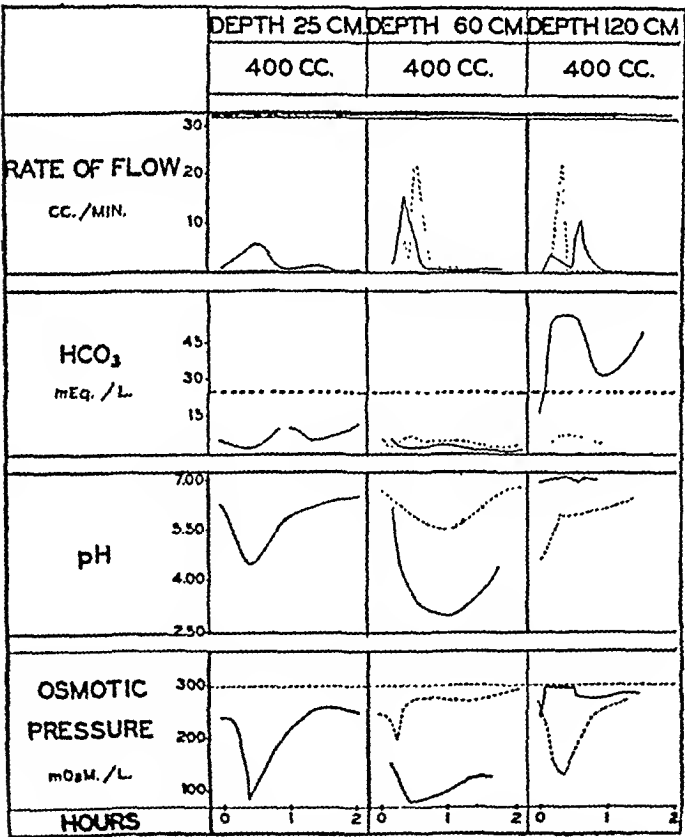


FIG. 4. CURVES TO SHOW THE EFFECTS OF THE ORAL ADMINISTRATION OF WATER ON THE CONTENTS FROM FIVE FASTING SUBJECTS.

Each subject is represented by a different type of line. The water was administered at zero time.

In four out of five cases, as shown by the prompt increase in the rate of flow, the water left the stomach rapidly. With this sweeping out of the basal acid secretions of the stomach the pH of the intestinal fluid decreased. The osmotic pressure

of the fluid also was lowered until approximate equilibrium was established after a variable interval. In one case in which only a slightly increased rate of flow occurred within the first half hour, the influence of the alkaline intestinal fluid predominated as indicated by the increase in bicarbonate, the higher pH and the maintenance of isotonicity.

DISCUSSION

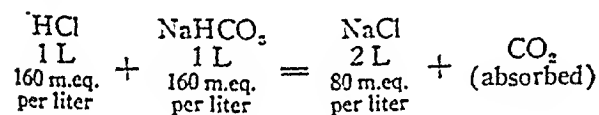
The data covered in this report are based entirely on a chemical study of intestinal contents as they normally occur and not of pure intestinal secretion. Chemical analyses of intestinal juices per se, without an admixture of substances from above, secured by the use of a three-lumened double-ballooned apparatus (12) that allows the fluid to be collected from an isolated segment of bowel, will be reported separately by Abbott and Karr (13).

Since the contents at any one point are a mixture of saliva, gastric juice, bile, pancreatic secretion and succus entericus originating above the point of collection, the composition depends primarily upon the ingredients of these separate secretions and secondarily upon the chemical reactions that occur among them. Gastric juice is hypotonic with respect to blood plasma. The acid gastric juice enters the duodenum and there comes in contact with secretions of alkaline reaction and of isotonic composition (8). The fasting contents of the small intestine beyond the duodenum are essentially a mixture of two fluids, gastric and duodenal, and they have a tendency to assume the characteristics of whichever may predominate at the time.

The great variation in the pH of the fasting intestinal contents is associated with a marked variation in the motility of the stomach and the intestine and, to a less extent, with a variation in the acidity of the fasting stomach contents and in the amount of pancreatic secretion. If the acid gastric juice while fasting leaves the stomach rapidly and is carried down the intestine by active peristalsis, the pH at any level tends to be low. If the progress of the contents is slower, complete neutralization occurs and sometimes, when intestinal secretions are in excess, an alkaline reaction results. Roentgenological examination supports

this relationship to motility. Such observations as apply to the duodenum are in keeping with the results of many similar studies previously made on man. With regard to the jejunum and ileum, only McClendon and his associates (14) have recorded the reaction in intact subjects, none of whom was in the fasting state. The most nearly comparable studies on animals are those of Mann and Bollman (15), who have showed in fistula dogs that the reaction of the intestinal content varies approximately in the same manner as that reported in this paper.

The studies of osmotic pressure of the intestinal contents show a wide variation from hypotonicity to practical isotonicity with the blood plasma. Two factors tend to produce a low osmotic pressure in the duodenum: the presence of hypotonic solutions from the stomach and neutralization. The following equation shows the neutralization of isotonic hydrochloric acid by isotonic sodium bicarbonate:



The carbon dioxide is very readily absorbed (16) and the resultant state of hypotonicity persists until equilibrium is established by the absorption of water or the diffusion of salt into the fluid. In any event osmotic equilibrium is usually attained, although if motility is very rapid, fluids with an acid pH and a low osmotic pressure may be found in the lower small intestine. When the motility is slow the fluids are neutral or alkaline and isotonic with blood plasma.

The bicarbonate content obviously varies with the hydrogen ion concentration. When the intestinal pH is in the neighborhood of 7.4 the bicarbonate content often approximates that of blood plasma. Variations are probably due to greater variations in the carbon dioxide tension. How much of the bicarbonate for neutralization comes from the pancreatic juice and how much through the intestinal wall is unknown. In the duodenum the pancreatic bicarbonate is probably the more important factor. In intestinal segments which have been blocked off, however, as will be shown in a subsequent presentation (13), certain stimuli may provoke a secretion of bi-

carbonate greatly in excess of its concentration in the blood plasma.

The chief function of the chloride ion in its reciprocal relationship to the bicarbonate ion would seem to be that of maintaining osmotic pressure. That it is present in the intestinal fluid in greater concentration than in blood plasma may not indicate a specific secretory action of the intestinal wall but rather an equilibrium of the only anion available to maintain osmotic pressure.

It is fully realized that the presence in the intestinal tract of the tube and the inflated balloon may disturb to some extent its normal chemical physiology. The variations in the chemical data on an individual intubated on different occasions, however, would seem to be due to functional variations beyond control and occurring in spite of the fact that the apparatus always, in our work, has been introduced and maintained under constant conditions.

#### SUMMARY

(1) A technique of intubation which makes practical the recovery of contents from approximately any desired portion of the intact human small intestine has been employed in a study of the chemical characteristics of the normal bowel under varying conditions.

(2) Under fasting conditions it has been demonstrated (a) that the flow of contents into any part of the small intestine is usually less than 1 cc. per minute; (b) that the acidity of the contents is greatest in the duodenum, diminishing gradually toward a neutral or even a slightly alkaline reaction in the lower ileum; (c) that the bicarbonate concentration is related to the reaction of the contents, and (d) that the duodenal contents if acid and in the process of neutralization are uniformly hypotonic, but that if neutral or alkaline they approach closely the osmotic state of the ileal contents which are essentially in equilibrium with the blood plasma.

(3) After the oral administration of a solution of hydrochloric acid isotonic with the body fluids, the contents of the stomach usually pass slowly into the duodenum, where they are completely neutralized by the bicarbonate content of the latter and attain osmotic equilibrium with the body.

(4) After the administration of an isotonic solution of sodium bicarbonate, a very rapid evac-

uation of the stomach occurs; but after a 5 per cent solution, the discharge is less rapid though still sufficient to produce distinctly alkaline and hypertonic duodenal and intestinal contents. After a 10 per cent solution, however, gastric retention occurs to such an extent that within two hours no increase in the bicarbonate content or alkalinity of the duodenal contents can be demonstrated.

(5) After the administration of 400 cc. of water, the gastric contents pass into the duodenum with sufficient rapidity to render the intestinal fluids acid, and to lower their osmotic pressure.

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# STUDIES OF UREA EXCRETION. IX. COMPARISON OF UREA CLEARANCES CALCULATED FROM THE EXCRETION OF UREA, OF UREA PLUS AMMONIA, AND OF NITROGEN DETERMINABLE BY HYPOBROMITE

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According to the usually accepted concept, the urea removed from the blood by the kidneys is excreted in two forms, chiefly as urea itself, but partly as ammonia. That the ammonia of the urine is formed in the kidneys was demonstrated by Nash and Benedict (1921), in work which has been confirmed by other authors (cf. Peters and Van Slyke, I, p. 373). Evidence from animal experiments *in vivo* has indicated urea as the probable chief source of the ammonia formed by the kidney (cf. Peters and Van Slyke (1931) p. 373). Krebs' recent work (1933) with kidney tissue *in vitro* has indicated the possibility that blood amino acids may also be a direct source of the ammonia formed by the kidney, but whether an important part of the ammonia actually excreted comes from them has not been demonstrated.

If urinary ammonia is entirely or chiefly formed from the blood urea, the urea cleared from the blood per minute by the kidneys is represented more accurately by the excretion of urea + ammonia rather than of urea alone. It appears possible, therefore, that urea clearances calculated from the excretion of urea + ammonia may measure the work of the kidneys in excreting urea from the blood more accurately than clearances calculated in the usual manner from the output of urea alone.

It therefore appears to be a problem of physiological interest to ascertain whether, when the ammonia:urea ratio in urine varies markedly, more consistent values for the urea clearance are calculated from the excretion rate of urea alone, or of urea + ammonia.

For clinical interpretation, as already pointed out by Bell, Gilmour and Cameron (1934), it would seldom make a serious difference if, in the formula for calculating the urea clearance (Möller, McIntosh and Van Slyke (1928, a)), urine

urea nitrogen were replaced by urea + ammonia nitrogen. Since the ammonia nitrogen ordinarily equals 1 to 10 per cent of the urea nitrogen, the clearance calculated from urea + ammonia would be from 1 to 10 per cent higher than that calculated from the urea excretion alone. In nephritis the proportion of nitrogen excreted as ammonia is likely to be less than in normal subjects, because the damaged kidney turns a smaller proportion than normal of the urinary nitrogen into ammonia (see literature quoted by Peters and Van Slyke (1931), p. 378; also data in Table III, present paper).

Conditions do occur, however, in which the ammonia:urea ratio may be unusually high. Such are the acidoses of diabetes and starvation, and the depression of urea output caused by very low protein, high calory, diets. If the urea clearance is measured in such a condition, it is of interest to know whether more consistent values may be expected when the calculation is based on the output of urea, or of urea + ammonia.

Aside from this, and the physiological interest of the problem, the question has some practical bearing on the convenience of procedures for determining the urea clearance. If a method for determining urinary urea is used which depends upon estimation of the ammonia formed when the urea is hydrolyzed by heat or urease, it is simpler to determine urea + ammonia than urea alone, since thereby one avoids an extra operation to remove or determine the preformed ammonia. Likewise, if the hypobromite gasometric method is used, it is simpler to determine urea + ammonia, since the reagent gives practically the same yields of  $N_2$  from both. Furthermore, if, by bladder infection or delay in analyzing urine, part of the urea is decomposed into ammonia before analysis, no error is introduced into the figure representing the sum of urea + ammonia.



We have sought to solve the proposed problem by ascertaining whether, when the ammonia:urea ratio in the urine is greatly increased, calculation of urea clearances with, or without, inclusion of the excreted ammonia gives values more consistent with those yielded by the same subjects under ordinary conditions. In order to obtain maximal increase in the ammonia:urea ratio, the numerator was increased by inducing acidosis with ammonium or calcium chloride administered by mouth, while the denominator was decreased by putting the subjects on low protein diet. By these means the ratio could be raised from its average usual value of about 0.05 to as high as 1.0. Unless such means are taken to increase the ratio, the effect of adding the ammonia to the urea for calculation of the clearance is likely to be small (averaging about 5 per cent) in comparison with normal spontaneous variation in the clearance, which may be  $\pm 20$  per cent, so that it would be difficult to ascertain, except by statistical data, whether the inclusion of the ammonia increased or decreased the constancy of the clearance.

We have also determined the clearances of urea with and without inclusion of the urinary ammonia in normal subjects and nephritics on ordinary diets, in order to obtain data, in addition to those of Bell, Gilmour and Cameron (1934), indicating the usual range of the effect of including the ammonia.

Finally, led by the extreme rapidity and simplicity of the approximate gasometric determination of urea + ammonia with hypobromite, we have, in a series of nephritic subjects with renal function varying from normal to that of terminal nephritis, compared urea clearances with "hypobromite nitrogen" clearances. These were calculated by substituting, in place of the urine urea nitrogen in the clearance formula, the urinary nitrogen determined by the hypobromite method, which is approximately  $0.95 \times$  (urea nitrogen + ammonia nitrogen).

#### METHODS

*Blood urea* was determined by the gasometric urease method (Van Slyke, 1927). *Urea in urine* was determined by the gasometric urease method (Van Slyke, 1927). *Ammonia in urine* was determined by the aeration technique of Van Slyke and Cullen (1914, 1916).

*Creatinine* in blood and urine was determined by the methods of Folin (Peters and Van Slyke, 1932, pp. 600 to 604).

For the *approximate hypobromite determination of ammonia + urea in urine*, the manometric procedure of Van Slyke (1929) was used, with the modification that the removal of ammonia by treatment with permittit was omitted. The urine was simply diluted 10- or 20-fold according to its concentration, and a sample was transferred to the manometric apparatus for the analysis. The improved hypobromite reagent of Van Slyke and Kugel (1933) was used. Twelve to 15 determinations could be done in an hour, and the procedure is so simple that the loss of an analysis, or failure of duplicates to check within 0.5 per cent, was a rarity.

#### CALCULATIONS

*Urea clearance.* The urea clearance was calculated by Formula 1 or 2, depending on the urine volume, as described by Möller, McIntosh and Van Slyke (1928, a) (see also Peters and Van Slyke (1932), pp. 564 to 570)

$$(1) C_{Ur} = \frac{1.33 UV}{B}, \text{ when } V > 2.$$

$$(2) C_{Ur} = \frac{1.85 U\sqrt{V}}{B}, \text{ when } V < 2.$$

$C_{Ur}$  = urea clearance, in per cent of average normal;  $U$  = urine urea nitrogen, in mgm. per 100 cc.;  $B$  = blood urea nitrogen, in mgm. per 100 cc.;  $V$  = urine volume in cc. per minute.  $V$  was corrected for body size, as described by McIntosh, Möller and Van Slyke (1928). (See also Peters and Van Slyke (1932), p. 569.)

*Clearance of urea + ammonia,  $C_{Ur+NH_3}$ .* The clearance of urea + ammonia, also in percentage of average normal urea clearance, was calculated by Formulae 1 and 2, but with  $U$  representing urea +  $NH_3$  nitrogen, instead of only urea nitrogen, in the urine.

*Hypobromite nitrogen clearance,  $C_{Hv}$ .* This clearance was calculated by the same formulae, but with the urinary nitrogen determined by the hypobromite method substituted for  $U$ . In this case  $U$  represents approximately 95 per cent of the urea + ammonia nitrogen.

*Creatinine clearance* was determined after giving 5 grams of creatinine, the calculation being like that of urea clearance by Formula 1, with 0.68 replacing the factor 1.33.

#### RESULTS

*Observations in which the ammonia:urea ratio in the urine was increased by induced acidosis and low protein diet.* It is obvious from Table I, dates April 23 to April 26, and Table II, dates June 7 to June 11, that when an important part of the urea + ammonia nitrogen was in the form of ammonia, the clearances calculated from the excreted urea alone were much below the usual urea clearance shown by the same subject. When the clearances were calculated from the excretion

TABLE I

Comparison of clearances of urea and urea + ammonia in subjects before and during induced acidosis. Subjects with normal renal function

Date	Protein in diet	Acidifying salt taken	Urine volume corrected for body size	Urine urea nitrogen. Urease method	Urine ammonia nitrogen. Van Slyke-Cullen aeration	Blood urea nitrogen. Urease on whole blood	Urea clearance $C_{Ur}$	Urea + $NH_3$ clearance $C_{Ur+NH_3}$	Ratio of $C_{Ur+NH_3}$ to $C_{Ur}$	Excretion rate of urea + $NH_3$ calculated as nitrogen	Venous plasma $CO_2$
		grams	cc. per minute	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	per cent of normal	per cent of normal $C_{Ur}$		grams per 24 hours	mM. per liter
February 26	Unlimited	0	4.6 9.3	185 92		10.2 10.2	112 112				
March 17	Unlimited	0	5.4 2.7	166 280		12.8 12.8	93 80				
March 19	Unlimited	0	1.9 1.9	435 401	14.4 10.2	12.8 12.8	87 81	90 83	103 102	12.2 11.2	
March 29	Unlimited	$NH_4Cl$ †	2.8 4.5	251 177	45.7 30.4	12.8 12.8	74 85	88 100	118 117	12.0 13.4	22.1
March 30	Unlimited	$NH_4Cl$	3.5 5.1	235 150	51.6 31.2	12.9 12.9	84 80	103 96	122 120	14.4 13.2	20.0
April 2	Unlimited	$NH_4Cl$	1.8 1.7	329 354	96.7 104.5	12.2 12.2	68 70	88 91	129 129	11.1 11.4	15.4
April 16	20*	0	3.4 2.8	104 114	7.3 7.5	6.6 6.6	70 65	75 69	107 107	5.4 4.9	
April 23	20	$CaCl_2$ ‡	1.4 1.0	123 148	34.8 52.4	5.1 5.1	53 55	69 74	128 135	3.2 3.0	24.2
April 25	20	$CaCl_2$	4.2	34.5	27.0	3.8	50	90	180	3.7	
April 26	20	$CaCl_2$	3.8 2.5	42.8 53.1	35.9 54.0	4.3 4.3	50 42	93 85	186 202	4.1 2.8	18.3

\* Diet with only 20 grams protein, April 15 to 25, inclusive. Total calories 2300.

† Administration of 10 grams  $NH_4Cl$  daily, March 27 to April 12, inclusive (= 187 m.eq.).

‡ Administration of 10 grams  $CaCl_2 \cdot 2H_2O$  daily, April 21 to 26, inclusive, equivalent to 7.55 grams  $CaCl_2$  (= 137 m.eq.).

of ammonia + urea nitrogen, on the other hand, the  $C_{Ur+NH_3}$  values were within or near the range of clearances obtained from the subject under ordinary dietary conditions. The creatinine clearances (Table II) indicated, like the ammonia + urea clearances, no significant decrease of renal function during the periods of high ammonia:urea ratios in the urine.

It therefore appears that, when acidosis increased ammonia excretion until it was important in comparison with urea excretion, the state of renal function was more accurately indicated by the urea clearance calculated on the assumption that the ammonia + urea of the urine represent the urea cleared from the blood. Calculation on the basis of excreted urea alone in this condition gave clearance values which were abnormally low for the subjects.

Comparison of clearances of urea and of urea + ammonia in normal subjects and nephritics, without induced acidosis. Table III gives the results obtained from a series of subjects on ordinary diets. The nephritic patients are divided into two groups, according to whether their clearances were above or below 20 per cent of average normal. It is evident that the ammonia:urea ratio in the urine tends to decrease as renal destruction becomes greater. The ammonia nitrogen averaged 5 per cent of the urea nitrogen in the normal subjects, 3.5 per cent in those nephritics who showed clearances above 20 per cent of normal, and only 1 per cent in the terminal cases, with clearances less than 10 per cent of normal. The highest ammonia:urea nitrogen ratio in the entire series was 1.09 in normal subject Number 2.

TABLE II

Comparison of clearances of urea, urea +  $\text{NH}_3$ , hypobromite nitrogen, and creatinine, before and during induced acidosis. Subject G. Hypertension with normal clearance

Date	Protein in diet	Acidifying salt taken	Urine volume corrected for body size	Nitrogen per 100 cc. urine			Urea nitrogen per 100 cc. blood by urease on whole blood	Clearances, calculated in percentages of mean normal urea clearance			Plasma creatinine clearance	Excretion rate of urea + $\text{NH}_3$ nitrogen. Average of 2 periods	Venous plasma $\text{CO}_2$
				$\text{NH}_3$ by Van Slyke-Cullen aeration	Urea by urease	Hypobromite nitrogen		$C_{Ur}$	$C_{Ur+NH_3}$	$C_{Hy}$			
	grams per 24 hours		cc. per minute	mgm.	mgm.	mgm.	mgm.	per cent	per cent	per cent	per cent*	grams per 24 hours	mM. per liter
No acidosis													
May 30	<i>Ad. lib.</i>	None	1.21 3.55	22.1 6.1	892 341	885 342	19.9	91 81	94 83	91 82		16.9	
May 31	<i>Ad. lib.</i>	None	4.09 4.44	8.4 4.3	269 143	253 147	16.1	91 53	94 54	86 54	88 53	12.9	
June 1	<i>Ad. lib.</i>	None	8.08 2.22	4.4 12.4	163 421	165 419	15.2	116 82	119 85	117 82	101 79	11.7	
June 2	<i>Ad. lib.</i>	None	8.88 4.78	3.5 2.5	77 114	78 108	8.5	107 85	112 87	109 81	108 72	9.3	
June 3	20	$\text{NH}_4\text{Cl}$	9.06 4.44	3.6 4.1	59 115	62 120	7.9	90 86	95 89	95 90	119 95	8.0	
Acidosis													
June 5	20	$\text{NH}_4\text{Cl}$	4.80 3.38	20.4 28.6	109 151	126 175	9.5	73 71	87 85	85 83	106 75	8.9	20.4
June 7	20	$\text{NH}_4\text{Cl}$	4.63 4.09	29.1 30.1	99 92	122 116	11.2	55 44	71 60	68 57	93 57	7.9	17.8
June 10	20	$\text{NH}_4\text{Cl}$	1.16 3.20	175.9 64.2	373 163	522 212	11.1	67 63	99 88	94 83	105 74	9.8	16.4
June 11	20	$\text{NH}_4\text{Cl}$	1.96 1.69	116.7 130.3	237 286	340 403	12.3	61 56	74 81	71 79	81 66	10.1	
Mean and standard deviation of clearances May 30 to June 3								88 $\pm 15$	91 $\pm 16$	89 $\pm 15$	89 $\pm 21$		
Mean and standard deviation of clearances June 5 to June 11								61 $\pm 10$	81 $\pm 12$	78 $\pm 11$	82 $\pm 18$		

\* For plasma creatinine clearance 148 cc. per minute is taken as 100 per cent of mean normal.

If, in calculating the  $C_{Ur+NH_3}$  values, the factors 1.33 and 1.85 in Formulae 1 and 2 were reduced by 5 per cent, to 1.26 and 1.76 respectively, the  $C_{Ur+NH_3}$  values, calculated from excretion of urea + ammonia, would in no case in Table III deviate by more than 4 per cent from the true  $C_{Ur}$  values, calculated from the excretion of urea alone.

Comparison of the urea clearance with the clearance of nitrogen determinable by hypo-

bromite, in nephritic patients with varying degrees of renal impairment. Table IV shows that, in the 54 urines analyzed, the nitrogen determinable by hypobromite in the urine averaged exactly the same as the urea nitrogen determined with urease. In all but 2 urines the two methods of analysis gave within 5 per cent the same values. In one case the hypobromite method gave 6 per cent more, and in one 12 per cent more than the urea nitrogen.

These are the results to be expected. As shown in the original description of the manometric hypobromite method (Van Slyke (1929)),

TABLE III

*Comparison of clearances of urea and of urea + ammonia in normal subjects and in nephritics with varying degrees of renal impairment*

Urea in blood and urine by gasometric urease-CO<sub>2</sub> method (Van Slyke (1927)). Ammonia in urine by aeration and titration (Van Slyke and Cullen (1914)).

Number	Initials	Urea clearance $C_{Ur}$	Urea + NH <sub>3</sub> clearance $C_{Ur+NH_3}$	Ratio of $C_{Ur+NH_3}$ to $C_{Ur}$
		<i>per cent of normal <math>C_{Ur}</math></i>	<i>per cent of normal <math>C_{Ur}</math></i>	
Normal subjects				
1	E.K.	130 164	137 168	1.06 1.03
2	B.	65 100 101	72 102 110	1.06 1.02 1.09
3	J.M.	118 117 111	125 124 115	1.06 1.06 1.03
Average				1.05

Nephritic patients with clearances above 20 per cent

1	A.Ch.	72 70 83	74 72 85	1.03 1.02 1.03
2	A.Co.	53.6 55.4 37.1† 32.6† 44.6‡ 23.3‡	55.0 56.0 38.0 33.1 47.4 24.8	1.03 1.01 1.02 1.02 1.06 1.06
3	McG.	75.9 48.4 31.8 32.6 32.0 28.4	77.1 51.5 34.4 35.1 34.0 29.4	1.01 1.06 1.08 1.08 1.06 1.04
4	Ra.	28.4 38.9	28.9 39.9	1.02 1.02
5	Cal.	29.1 24.5	29.4 24.8	1.01 1.01
6	Or.	18.7 22.0	19.5 22.6	1.04 1.03
Average				1.035

† Low protein diet, 20 grams per day.

‡ High protein diet, 130 grams per day.

TABLE III—continued

Number	Initials	Urea clearance $C_{Ur}$	Urea + NH <sub>3</sub> clearance $C_{Ur+NH_3}$	Ratio of $C_{Ur+NH_3}$ to $C_{Ur}$
		<i>per cent of normal <math>C_{Ur}</math></i>	<i>per cent of normal <math>C_{Ur}</math></i>	
Nephritic patients with clearances below 20 per cent				
1	Fl.	9.00 8.30 7.70 8.20 9.20 9.10	9.20 8.30 7.80 8.40 9.40 9.20	1.02 1.00 1.01 1.02 1.02 1.01
2	Car.	8.65 8.65 9.65	8.78 8.78 9.78	1.01 1.01 1.01
3	Jo.	7.50 7.20	7.67 7.39	1.02 1.03
4	Wein.	5.79 5.79	5.88 5.91	1.02 1.02
5	Cap.	5.30 6.88 5.20 3.69 3.74	5.41 6.92 5.24 3.70 3.75	1.02 1.01 1.01 1.00 1.00
Average				1.01

TABLE IV

*Comparison of clearances of hypobromite nitrogen and of urea nitrogen in nephritic patients with varying degrees of renal impairment*

Number	Ratio of urea nitrogen to hypobromite nitrogen in urine*	Urea clearance $C_{Ur}$	Hypobromite nitrogen clearance $C_{H_2}$
		<i>per cent of normal <math>C_{Ur}</math></i>	<i>per cent of normal <math>C_{Ur}</math></i>
1	1.01 1.01	151 129	153 130
2	1.01 1.00	188 110	190 110
3	0.96	106	103
4	1.04 1.04	112 112	117 117
5	1.02 0.97	90 107	92 104
6	0.94 0.97	93 84	88 82
7	1.00 0.99	83 65	83 64

\* This urine concentration ratio is also the clearance ratio,  $C_{Ur}:C_{H_2}$ .

TABLE IV—*continued*

Number	Ratio of urea nitrogen to hypobromite nitrogen in urine*	Urea clearance $C_{Ur}$	Hypobromite nitrogen clearance $C_{Hy}$
		<i>per cent of normal <math>C_{Ur}</math></i>	<i>per cent of normal <math>C_{Ur}</math></i>
8	0.97	89	85
	0.97	71	69
9	0.96	87	84
	1.02	67	68
10	1.01	95	96
	1.00	75	75
11	0.98	72	71
	0.94	73	69
12	1.01	69	71
	1.00	69	69
13	1.05	55	58
	1.12	56	63
14	1.01	47	47
	1.02	57	58
15	0.98	48	47
	0.99	41	41
16	0.98	44	43
	1.01	40	40
17	0.97	30	30
	0.96	33	30
18	1.05	26	27
	1.02	37	38
19	0.99	27	27
	1.00	33	33
20	1.00	23.1	23.0
	1.03	22.4	23.1
21	0.97	15.8	15.4
	0.97	18.6	18.1
22	1.01	17.2	17.3
	0.98	16.0	15.8
23	0.97	15.3	14.8
	0.98	15.9	15.6
24	1.01	13.3	13.4
	1.03	14.1	14.5
25	1.02	12.8	13.0
	1.02	14.2	14.4
26	1.00	14.6	14.6
	1.01	10.3	10.4
27	0.95	9.4	8.9
	1.06	10.2	10.8
28	0.98	2.2	2.2
Average ratio	1.00		

the nitrogen yielded by it from urea, under the conditions prescribed for urine analysis, is 95 per cent of theoretical. With ammonia the yield is almost the same, 96 per cent (Van Slyke (1927)). As the ammonia nitrogen in the urine ordinarily averages about 5 per cent of the urea nitrogen (see for example Table II), one could expect that, when the hypobromite reagent is applied with the urinary ammonia present, the  $N_2$  gas yielded by the ammonia would, on the average, make up the 5 per cent deficit in the  $N_2$  yielded by the urea.

Hypobromite clearances less than 95 per cent of the urea clearance cannot occur, except as the result of analytical error, because the hypobromite reagent gives 95 per cent of theoretical  $N_2$  from pure urea. (It also evolves some  $N_2$  from other urinary substances, such as uric acid and creatine, but their effect on the result in human urine appears to be ordinarily slight.)

Hypobromite nitrogen clearances above 105 per cent of the urea clearance will occur when the ammonia nitrogen exceeds 10 per cent of the urea, e.g., Number 13, Table IV. In such cases the hypobromite nitrogen clearance will approximate (95 per cent or a little more) the clearance of urea + ammonia nitrogen, which has been shown above to be a more consistent measure of renal function than the clearance of urea alone.

It appears that the comparative significance of the urea clearance and the hypobromite nitrogen clearance may be summarized as follows.

1. The average normal values yielded by both are identical, hence the same standard normal values, heretofore used for the urea clearance, apply without change to the hypobromite nitrogen clearance. In calculating percentages of normal values for the hypobromite nitrogen clearance, the factors 1.33 and 1.85, of Formulae 1 and 2, can therefore be used without change, also the nomograms for graphic calculation of the clearance. (Möller, McIntosh and Van Slyke (1928, a); Peters and Van Slyke (1932), pp. 566 and 567.)

2. The hypobromite nitrogen clearance will always be 95 per cent or more of the urea clearance.

3. When, by reason of an unusually high ammonia:urea ratio in urine, the hypobromite nitrogen clearance significantly exceeds the urea clearance, the hypobromite nitrogen clearance may be

taken as the more accurate measure of renal function, since it approximates the urea + ammonia clearance, which has been found to be more consistent than the simple urea clearance.

From these conclusions it appears that the hypobromite nitrogen clearance can be taken not only as a technically convenient, but also as an accurate measure of renal function, practically equalling for this purpose the true urea clearance when the ammonia:urea ratio in the urine is within ordinary limits, and excelling the urea clearance when the relative ammonia excretion is exceptionally great.

#### *Present urea clearance procedure in this Clinic*

Experience accumulated since the details for determining the urea clearance were first published (Möller, McIntosh and Van Slyke (1928, a)) has somewhat more completely indicated the conditions desirable for the procedure, and we will briefly outline the manner in which it is now carried out.

*Preparation of the subject.* Except in fairly advanced cases of nephritis, it ordinarily makes no significant difference whether the subject lies down during the test or walks about. Van Slyke, Alving and Rose (1932) found that it made no difference in any subject examined by them who showed more than 50 per cent of normal clearance, but that 3 out of 12 nephritics with less than 50 per cent normal function showed lower clearances if they were up and about. It is therefore desirable that nephritics, who have advanced to less than 50 per cent of normal clearance, rest in a reclining posture during the test, but in other subjects it is not essential.

It is desirable to promote a fairly free flow of urine during the test, in order to diminish the relative error caused by retention of urine in the bladder at the voidings. For this purpose we routinely give two glasses of water, one at the beginning of the test, and another an hour later, after the first specimen of urine has been collected. This is the only special preparation of the subject which we routinely follow. It may be omitted when for any reason it is desirable to do so.

The gain in constancy of results caused by increasing the urine volume is indicated by the following results.

Möller, McIntosh and Van Slyke (1928, a) found that the mean probable deviation, in a given subject, from the average clearance of that subject, was  $\pm 9.2$  per cent when the urine volume was under 2 cc. per minute, and  $\pm 7.0$  per cent when the volume exceeded 2 cc. Addis (1922) obtained routinely a great diuresis by giving one liter of water, with 15 grams of urea, 3 hours before the test, and two glasses (about 400 cc.) each hour thereafter until the test was completed. Under these conditions the mean probable deviation of the clearance in an individual was reduced to  $\pm 3.7$  per cent.

Some of the earlier studies of urea excretion indicated that coffee affected the results (Addis and Drury (1923)), and that clearances taken in the morning might be more consistent than those taken at other times of the day. Page (1933) has found, however, that the amount of caffeine in an ordinary cup of coffee has no measurable effect on the clearance. And general experience in this hospital has shown that the time of day also has no significant effect. The clearance may therefore be taken whenever it is most convenient, and it is not necessary to forbid the taking of a single cup of coffee or of ordinary amounts of food before the test.

Addis and Watanabe (1916) and Addis (1922) gave 15 grams of urea routinely, except in cases with blood urea already high, with the idea that the increased load of urea would put the kidney under a strain and reveal functional deficits that might otherwise be missed. Fowweather (1934) also recommends urea administration on the basis of his experience, but for exactly the opposite reason; giving the urea appeared to prevent an occasional erroneously low clearance which was encountered when urea was not given. Cope (1934), apparently influenced by Fowweather's results, advises the giving of urea. Möller, McIntosh and Van Slyke (1928, a and b), however, found that the urea administration had no influence, either in raising or lowering the clearance, either in normal or in nephritic subjects. Later, Van Slyke, Alving and Rose (1932) again tested the question carefully, and likewise concluded that urea administration was without effect on the clearance values. It is the writers' belief that the administration of urea increases the accuracy of the clearance determinations only when, by increasing the blood urea content (Fowweather found an average increase of 16 mgm. urea nitrogen per 100 cc. blood after giving 15 grams of urea), it augments the accuracy of the blood urea determinations. When a technique for blood urea is used which gives satisfactorily accurate results with the concentrations of blood urea naturally present, there appears to be nothing gained by urea administration. It complicates the procedure, and the methods for rapid blood analysis now available are sufficiently accurate to make the addition unnecessary. One of the advantages of the urea clearance as a renal test is that it is not necessary to complicate it by administration of anything.

*Collecting the urine.* It is desirable to collect two specimens of urine, each over a measured

period, for which about an hour is a satisfactory time. A single specimen of blood for analysis drawn near the middle of the two-hour interval serves for comparison with both urine specimens. In consequence, two clearances are measured with only one drawing of blood.

To start the first period, the subject, after drinking his first glass of water, empties his bladder completely, without saving the specimen, and a stopwatch is started. At the end of approximately an hour the subject again voids completely and the specimen is saved (Period 1).

At the moment when the subject finishes voiding, the time on the stopwatch is recorded, as the first period, and the watch is immediately restarted for the second period. The subject then drinks his second glass of water.

At approximately the end of the second hour he again voids completely, the time of this period is accurately recorded, and the specimen is saved (Period 2).

It is not necessary that the time of each period shall be exactly an hour; it may without harm be longer, up to several hours; or it may be shorter than an hour, provided enough urine (preferably over 50 cc.) is voided to prevent undue error from the volume retained. The essential things are, that the time be exactly recorded, that the approximately complete urine content of the bladder be obtained, and that the blood urea determined represent approximately the average for the period.

In some patients it is impossible to obtain urination at will. In such cases one may continue the collection period for several hours until spontaneous urination occurs, provided the time is accurately recorded. Especially in young children, it is frequently necessary to wait for spontaneous urination. For these the automatic recording device described in the accompanying paper by Farr (1935) is a great advantage.

*Urine measurement.* The urine from each period is measured within 1 per cent. Accurate graduated cylinders of 25, 50, 100 and 200 cc. are kept at hand, and the smallest is used which will contain the specimen.

*Collecting blood.* At about the end of the first hour of the usual 2-hour test, a sample of blood is drawn for analysis. Ordinarily, when no urea has been administered, the change of blood urea

content is slow, so that a matter of 10 or 15 minutes time, or even more, sooner or later, is not important. We usually draw 2 cc. or more of blood from a vein into a syringe, thereby obtaining sufficient for duplicates by the usual gasometric methods. When, however, economy of blood is desirable, 0.2 cc. is taken from a finger or ear, or, for small children from the heel as described by Drucker and Cullen (1925), and the urea is determined by micro-analysis.

*Urine analysis.* The rapid hypobromite urea method with the manometric apparatus is used (Van Slyke (1929), Peters and Van Slyke (1932), p. 379). The *treatment with permittit to remove ammonia*, included in the original description of the method, is omitted for reasons developed in this paper. The urine is merely diluted 10 or 20-fold, a sample is measured into the manometric chamber, hypobromite is added, the chamber is shaken 1.5, 2 or 3 minutes at 25°, 20°, or 15°, respectively, and the analysis is finished by taking the reading. The improved hypobromite reagent of Van Slyke and Kugel (1933) is used.

*Analysis of blood.* The manometric hypobromite method is also routinely used for blood urea. It is applied, as described by Van Slyke and Kugel (1933), to the blood filtrate obtained by deproteinization with zinc hydroxide according to the procedure of Somogyi (1930). The correction of 1.2 mgm., recommended by Van Slyke and Kugel for N<sub>2</sub> evolved from non-urea substances in the Somogyi blood filtrate, is applied. As found by Van Slyke and Kugel, the maximum error by this method is 0.5 mgm. of urea nitrogen per 100 cc. of blood. The method is simpler with regard to reagents and procedure than the urease methods, and is as accurate as the urease procedure recommended for routine analyses ("Procedure A, for determinations in a series of blood filtrates," p. 703, Van Slyke (1927)).

For micro-analyses of blood drawn from skin puncture, the micro-gasometric urease method (Van Slyke (1927)) has until lately been employed. However, a micro-hypobromite modification has been developed by Farr, and is described in his accompanying paper (1935).

*Calculation.* When the urinary hypobromite nitrogen is determined as above described, the clearance is calculated by Formulae 1 or 2, with

the urine nitrogen determined by the hypobromite representing  $U$  in the formulae. The same nomograms (Möller, McIntosh and Van Slyke (1928, a); Peters and Van Slyke (1932), pp. 566 and 567) used for facilitating calculation of the clearance from exact urinary urea values are used also with the hypobromite nitrogen values.

If laboratory conveniences should make it easier to determine urea + ammonia nitrogen (e.g., by the urease method, with determination of the pre-formed ammonia together with the ammonia formed from urea) one would use the formulae with the constants 1.26 and 1.76 substituted for 1.33 and 1.85.

*Clearance by direct colorimetric comparison of blood and urine urea.* (Van Slyke and Cope (1932).) This method is convenient where laboratory facilities make colorimetry more practicable than manometric analysis. Because the reverse is the case in this laboratory, the colorimetric procedure has not been used routinely by the writers. However, it has given satisfactory routine service in the clinic of one of our colleagues (Prof. L. H. Newburgh).

In the light of results in the present paper, the colorimetric clearance may be simplified by omitting the preliminary treatment of the urine with permittit to remove ammonia, and by making the urine dilutions to 1.05 times the volumes indicated by the graph of Van Slyke and Cope.

#### SUMMARY

Experiments with human subjects show that when the proportion of urea in the urea + ammonia mixture of the urine is markedly decreased by induced acidosis and low protein diet, the urea clearances calculated from the excretion rate of urea alone suffer a parallel reduction. If, however, values for excretion of urea + ammonia are substituted for urea, the clearances calculated remain at the usual levels.

The theoretical significance of the results is to favor the hypotheses, that the ammonia excreted in the urine of man is formed in the kidneys chiefly from urea removed from the blood, and that the work of the kidneys in excreting urea from the blood is more accurately indicated by the combined excretion of urea and ammonia than by the excretion of urea alone.

The practical deduction is that in determinations of the urea clearance as a measure of renal function, results are somewhat more consistent if in the clearance formula,  $\frac{UV}{B}$  or  $\frac{U\sqrt{V}}{B}$ , one uses for  $U$  the urinary concentration of urea + ammonia nitrogen, instead of only urea nitrogen. When the urinary urea is determined by methods measuring the  $\text{NH}_3$  formed by urea hydrolysis, or the  $\text{N}_2$  yielded by the action of hypobromite, the determination of combined urea + ammonia nitrogen is also simpler than determining the urea nitrogen separately.

The routine procedure developed for clinical determination of the urea clearance is described.

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# A MICRO METHOD FOR BLOOD UREA AND AN AUTOMATIC URINE COLLECTOR FOR UREA CLEARANCE IN INFANTS

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## *Hypobromite micro method for blood urea*

The blood is precipitated according to the method of Steiner, Urban and West (1932), and the urea is determined manometrically by the hypobromite reaction, as applied by Van Slyke and Kugel (1933).

Twenty-five hundredths cubic centimeters of blood is laked in 12.0 cc. of distilled water which has been measured into a conical centrifuge tube with calibrated volumetric pipettes. The blood is stirred to ensure adequate mixing and 0.25 cc. of 17 per cent  $\text{FeSO}_4 \cdot \text{H}_2\text{O}$  is added and mixed thoroughly with a stirring rod. Then 0.2 to 0.4 gram of solid  $\text{BaCO}_3$  is added. This is most conveniently done by using a small ladle which has previously been measured to contain the correct weight of  $\text{BaCO}_3$ . An excess of  $\text{BaCO}_3$  does not injure the analysis. The mixture is thoroughly stirred until the precipitate begins to flocculate. Settling is rapid, and the supernatant liquid should be clear. If there is a yellowish tinge, some additional  $\text{BaCO}_3$  should be added and the contents thoroughly mixed. The tube is then centrifuged for 15 minutes at 2500 R.P.M. or more, and filtered through a small (5 or 7 cm.) filter paper into a 25 cc. Erlenmeyer flask. Usually 10.5 to 11.0 cc. of filtrate are obtained.

For the urea determination 5 cc. of filtrate are transferred to the chamber of the Van Slyke-Neill manometric apparatus by means of an Ostwald bulb pipette. The 5 cc. of filtrate represent 0.1 cc. of the original whole blood sample. The chamber is sealed, and is evacuated and shaken for two minutes to free the solution of dissolved gases. These are expelled, and 1.5 cc. of the hypobromite solution is run into the chamber, which is then sealed, evacuated and shaken for the necessary reaction time, about  $1\frac{1}{2}$  minutes, at ordinary room temperature. The pressure of the gas evolved is measured with the gas occupying a volume of 0.5 cc. The filtrate from 0.25 cc. of blood suffices for duplicate analyses.

An empirical correction of 1 mgm. urea nitrogen per 100 cc. blood is subtracted when the uncorrected value exceeds 10 mgm., but not when it is less than 10 mgm.

It is not necessary to remove the barium in determining the urea by this method. If 0.25 cc. of blood is not available it is possible to perform the analysis on as little as 0.1 cc. of blood, though the accuracy of the result is correspondingly lessened.

The results when 0.25 cc. of blood are used are accurate to within 5 per cent of the true value unless the blood urea nitrogen is below 10 mgm. per cent.

## *Infant urine collector and annunciator apparatus*

The following apparatus was developed in order that the rate of urine excretion in infants could be determined as exactly as in adults. This allows one to calculate the urea clearance, for which it is necessary to be certain that all of the voided urine is collected, and that the interval during which the urine is excreted is accurately timed.

A 1000 ml. wide-mouth bottle is used as the container for the apparatus (see Figure 1). This is kept clean so that any urine which escapes from the collection cup can be recovered and measured. The mechanical parts are fixed to an ordinary rubber stopper 5 cm. in diameter. Through this stopper in an eccentric position is led a  $\frac{3}{8}$  inch glass tube (*U*) which is curved after it passes into the bottle so that the lower opening is below the center of the cork and over or extending into the mouth of the 30 ml. beaker that is used for the collection of the specimen. The beaker is suspended from a spring arm (*C*), the suspending string passing through the center of the cork. It is connected with the beaker by a hook (*D*) which holds the beaker handle (*E*) which is connected to the beaker by being wound tightly about it. This system must be so arranged that the beaker swings freely. The diameter of the beaker is sufficiently less than that of the mouth of the

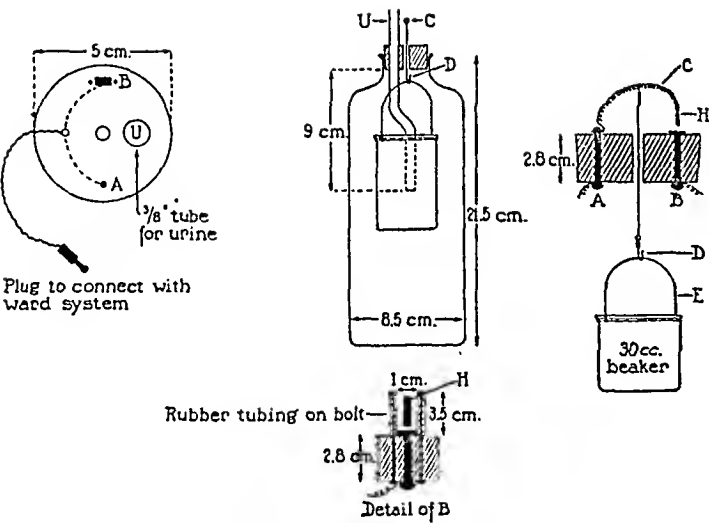


FIG. 1. DETAILS OF URINE COLLECTOR AND ANNUNCIATOR  
For symbols, see text.

TABLE I

Factors by which  $P_{N_2}$  is multiplied to give urea or urea nitrogen per 100 cc. of blood

Temperature °C.	Factors giving mgm. urea N	Factors giving mgm. urea
15.....	0.7960	1.703
16.....	0.7930	1.697
17.....	0.7900	1.690
18.....	0.7875	1.685
19.....	0.7845	1.679
20.....	0.7815	1.672
21.....	0.7790	1.667
22.....	0.7760	1.660
23.....	0.7730	1.654
24.....	0.7705	1.649
25.....	0.7680	1.643
26.....	0.7650	1.637
27.....	0.7625	1.632
28.....	0.7600	1.626
29.....	0.7575	1.621
30.....	0.7545	1.615
31.....	0.7520	1.609
32.....	0.7490	1.602
33.....	0.7465	1.597
34.....	0.7445	1.593

bottle to allow free withdrawal of the beaker when full. The alarm apparatus consists of a wire spring arm (C) to which the beaker string is fastened at about the middle. One connection from the wall plug (A) is connected to the base of this spring arm, the other is fastened to the contact (B). The wire itself is led through the cork, and the connections are made on the lower side of the cork. The spring contact is so adjusted that 2 to 3 cc. of urine will depress the beaker and arm sufficiently to make contact. On either side of the flat contact head is placed a long screw which is covered with rubber tubing. (See B in detail, Figure 1). This acts as a guide for

TABLE II

Analyses of blood showing comparisons with macro urease determinations

Sample	$P_{N_2}$	Temperature	Urea N per 100 cc.	Urea N per 100 cc. average value	Urea N per 100 cc. by macro urease method
	mm.	° C.	mgm.	mgm.	mgm.
$R_2$	10.5 10.8	27.5	8.00 8.20	8.1*	8.53
M	23.8 24.8	25.5	16.95 17.80	17.37	17.81
C	16.3 14.8	24.5	11.35 10.20	10.7	9.40
R	9.5 10.5	24.0	7.30 8.10	7.7*	8.05
P	207.5 210.0	27.0	157.10 158.80	157.9	160.20

\* Empirical correction of 1 mgm. not subtracted for urea nitrogen values below 10 mgm. per 100 cc.

the spring arm and ensures contact in the proper manner on all occasions. The two wires leading from the apparatus (as A and B) are connected to a plug which fits into the ward annunciator system. The urine is obtained from the child in the customary manner. On male infants the penis is inserted into an adapter tube of such a size as to avoid compression. This is held in place by a urinal binder, which consists of two strips of cotton material forming an X (see Figure 2). At the center of the X is made a hole which allows the adapter tube to slip through but which will not permit the lip of the tube to pass. The edges of the hole must be reinforced by buttonhole stitching to prevent tearing and stretching. The adapter is then held firmly in place by

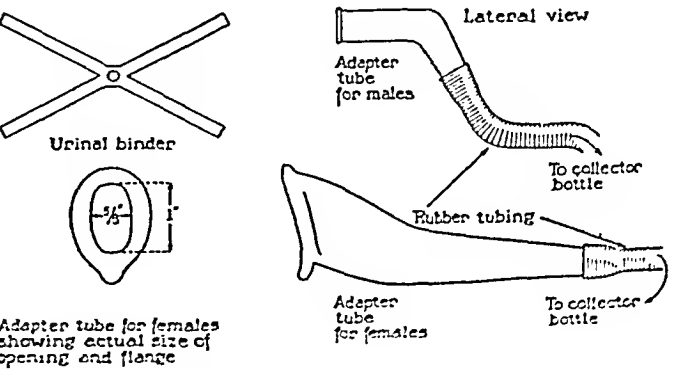


FIG. 2. DETAILS OF ADAPTER FOR URINE COLLECTOR

bringing the arms of the X around the child and pinning. For female infants the same binder may be used but a different shaped adapter tube is used. From the adapter tube the urine is led through a soft rubber tube directly over the foot of the bed to the alarm bottle. It is advantageous to raise the head of the bed and usually it is necessary to place restraints on the child's ankles. No other restraints are ordinarily required. Thus, when the child voids, the beaker is filled, depressing the spring arm and making contact. This rings the buzzer on the call board, and it is merely necessary to note the time in order to determine any series of intervals desired. The specimen of

urine in the beaker is collected, and the beaker replaced ready for the next specimen.

Clearances which have been calculated from data obtained in this fashion are comparable in every way to those done on older patients and on the same patient when voluntary control could be exercised.

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# THE METABOLISM OF THE ISOLATED HEART OF DOGS RELATED TO AGE<sup>1</sup>

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In attempting to understand disability of a chronic nature, more particularly in that group of ailments commonly designated as "degenerative diseases," it has become ever more apparent that description of the successive phases which healthy individuals exhibit during normal life is extraordinarily incomplete. A serious defect in knowledge accordingly exists which interferes in an important sense with the possibility of drawing conclusions concerning how far a particular healthy person differs from his fellows. In short the question as to whether his bodily state differs only so widely from the average as to fall within the range of chance biologic variation, or so widely as to lie outside and by so doing suggest the influence of some unknown agent must, for the most part, go unanswered.

A living organism is obviously the expression of the sum of the functions of its organs and structures, and these in turn depend for their behavior upon the units of which they are composed. It is well established (1), furthermore, that the growth and decay of the body and its parts follow curves of different rates of change when plotted against age. The thymus gland, for instance, reaches the peak of its development at puberty while the posterior molar teeth are not usually fully developed until the age of thirty. It follows, seemingly, that only by accumulating descriptions of change of the parts with age can change of the whole become known or understood.

The study of growth in human beings, especially of their organs and tissues, is beset with many difficulties. Their relative longevity, the complexity involved in unravelling the rôle of their inherited characteristics and the uncontrollability of environmental factors lead to variations so great as to tend to obscure the essential phenomena relevant to a description of ontogenetic

progress. In order to attempt an understanding of such processes the observer is forced to turn to the study of animals. The advantages of choosing, as has been done in this study, such an animal as pure-bred wire-haired fox terriers are apparent. They have a homogeneous ancestry, have a suitable size, and their ages can be known. Female specimens only were chosen. They may, furthermore, be furnished with similar food, exposed to the same climatic conditions and allowed to partake of similar physical activity. A certain simplification in the problem of managing the effect of environmental factors on aging is, in this way, achieved. Under these circumstances a variety of studies of dogs of this breed have been undertaken. The present communication deals with the relation of the metabolism of the heart, as indicated by the consumption of oxygen and elimination of carbon dioxide, to the age of the animal.

## METHODS

The method by which the heart and lungs were isolated from the rest of the body was essentially that of Knowlton and Starling (2). The operative technique need not be described except to mention the use of a knife in circuit with a high frequency current for cutting the soft tissue overlying the sternum. The time consumed in operating was, by this means, reduced materially. The amount of blood lost was negligible. The total capacity of the external circulation was 700 cc. The blood was warmed, and its temperature satisfactorily controlled during the time of its passage through a thin-walled rubber reservoir when the latter was immersed in water heated by an electric bulb thermostatically regulated. The level of the warm water around the reservoir was maintained uniform automatically (3) so that the venous pressure was constant.

The apparatus for analyzing respiratory gases differs in some particulars from most of those reported and is, therefore, described in greater detail. Starling's "Ideal respiration pump" was used for inflating the lungs. By means of three-way stopcocks one in the inspiratory and one in the expiratory tube, the respiratory system could be connected with the "closed" spirometer circuit (Figure 1). The air in the spirometer circuit was propelled at a rate of five liters a minute by a rotary pump through

<sup>1</sup> This paper is also No. 16 of the Series on Physiologic Ontogeny published from the Hospital of the Rockefeller Institute.

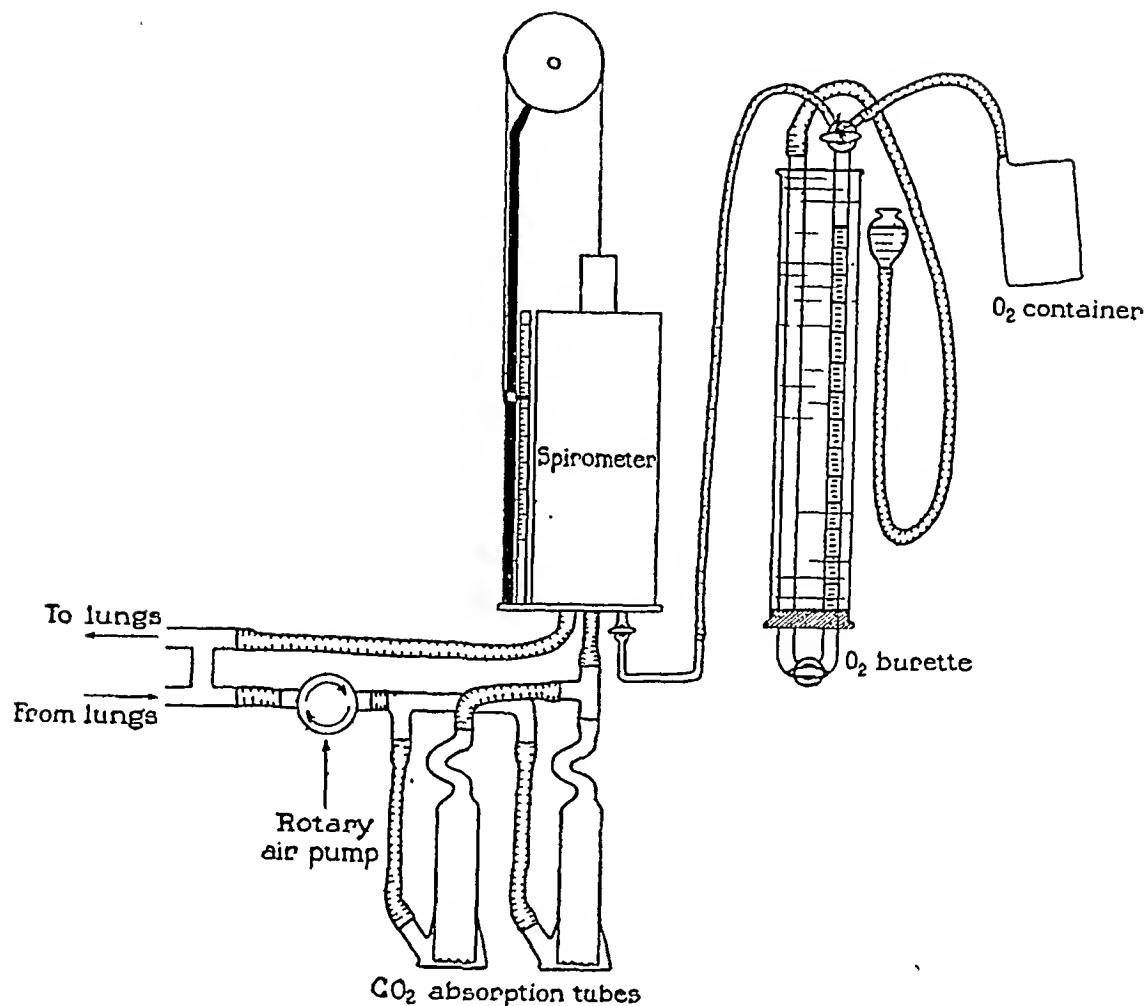


FIG. 1. A DIAGRAM OF THE APPARATUS USED FOR ANALYZING AND KEEPING UNIFORM THE MIXTURE OF RESPIRATORY GASES

See description in text.

one of a pair of receptacles each containing sodium hydroxide (20 per cent solution) to absorb carbon dioxide. The air then passed on into a spirometer and from there to the respiratory pump. The rate of movement chosen insured the passage onward of the expiratory air away from the inspiratory inlet before that valve opened.

The carbon dioxide excreted was absorbed in a known volume of sodium hydroxide solution, and the amount given off within a measured period of time (within 8 to 10 minutes) was calculated from the concentration of gas in the solution (4). The amount of oxygen consumed was calculated not from the fall of the spirometer, but from the amount it was necessary to add in order to keep it at the same level. This method was adopted because to maintain gaseous equilibrium between the blood perfusing the preparation and the respiratory air, it was essential that the preparation be supplied with air of constant composition. The quantity of oxygen added within a given time was measured in a calibrated burette of 100 cc. capacity from which it was displaced by water pressure into the closed respiratory system. The rate of passage was controlled by a screw clamp. By means of

an indicator the original level of the spirometer could be reproduced approximately within one-quarter of a millimeter, or 0.7 cc. Since the smallest volume of oxygen measured was 30 cc. the error involved in this manoeuvre was less than 2.4 per cent. The burette was easily read with an accuracy of 0.1 cc. so that the error from this source was not more than 0.3 per cent.

Changes in temperature introduced a small additional error. The volume of the respiratory circuit was one liter, and the rate of movement of air such that practically uniform temperature prevailed throughout the system. The greatest change encountered during any one measurement was  $0.2^{\circ}\text{C}$ . Complete neglect of change in temperature of the air in the lungs (roughly 200 cc.) would contribute an error of only 0.2 cc. and since the temperature of the circuit was ascertained to within  $0.05^{\circ}\text{C}$ . the error from this source was also 0.2 cc. The maximal error in correction of volume for changes of temperature was, accordingly, 1.2 per cent. The actual error was perhaps less than one-half this amount. All these errors combined constitute, at a maximum, a theoretical error of 4 per cent. It was found that duplicate measurements did not often differ by more than 3.5

per cent and that the average difference between duplicates was 2.5 per cent.

The animals were kept without food for 18 hours prior to operation. Anesthesia was induced by injecting chloralose 0.1 gram per kilo body weight, into any convenient vein anesthetized locally with novocaine. Other sedative drugs were not given. Not infrequently it was necessary to supplement the original dose of chloralose by as much as 0.05 gram per kilo. Rarely the dose was doubled. One fatality was obviously due to chloralose in over a hundred animals. Eighty-five consecutive experiments were carried out without the appearance of pulmonary edema except as an event associated with heart failure near the close of an experiment. In the eighty-sixth animal, however, this accident developed abruptly ten minutes after changing to the external or artificial circulation. This was the third successive experiment in which a small amount of ether by inhalation had been given as a substitute for an additional dose of chloralose. The practice was immediately discontinued, and in the remaining twenty-eight experiments pulmonary edema did not occur. Additional blood was obtained from a mongrel by bleeding from the carotid artery five minutes after heparin (10 grams per kilo) had been injected intravenously. The bleeding was carried out without anesthesia unless the animal became restless; ether was then administered.

After the circulation had been diverted to the artificial circulating system, a cannula connected to a water manometer was inserted into the right auricle through the inferior vena cava. The rubber tube from the arterial cannula was connected by a side arm to a mercury manometer. Plungers riding the liquid in the manometers wrote on a smoked drum.

The cardiac output was measured in a stromuhr brought into the circuit by turning a large glass cock, through which there were three passages—one, inlet and outlet, to each side of the stromuhr, and a by-pass. The borings are necessarily so arranged that on turning the cock from its central, by-pass, position, passage is provided for simultaneous flow into one side and evacuation of the other side of the instrument.<sup>2</sup> The output of the heart and the arterial pressure were adjusted to the desired levels.

Enough of a 10 per cent solution of glucose containing one unit of insulin (Lilly and Co.) for each gram of glucose was rapidly added to raise its concentration in the blood to between 200 and 250 mgm. per 100 cc. This usually required about a gram of glucose; the amount in the blood was estimated by the micro-time method of Hawkins (5). The glucose-insulin mixture was added mechanically at a uniform rate of 8 to 15 cc. an hour, depending upon the size of the heart. Often the rate chosen served to keep the concentration of glucose constant, but somewhat more frequently it rose slowly to a level of 300 to 350 mgm. glucose per 100 cc. of blood. The rate of injection was not changed, how-

ever, since it was observed that a reduction, or a sudden increase, in the inflow of the glucose-insulin mixture led to prompt changes in the efficiency of the heart.

About twenty minutes after changing to the artificial circulation, the respiratory system was connected to the spirometer circuit by turning the two 3-way cocks. Ten minutes were allowed to pass to permit the expiratory volume of the lungs to become adjusted to the slight difference in pressure existing between the respiratory and spirometer systems. Measurements of the consumption of oxygen and of the elimination of carbon dioxide were then begun in immediately successive periods of eight to twelve minutes. Constant values were obtained, usually within forty minutes, always within an hour. Unless signs of heart failure made their appearance, a fairly unchanging period in the life of the preparation was now established. Constancy of behavior was manifested in the uniformity of the cardiac output, of the venous and arterial pressures, as well as in the rate of metabolism. This was almost invariably the period of greatest efficiency lasting usually about an hour and not infrequently, two hours. Only those experiments in which a plateau of behavior like this persisted for at least a half-hour were included in the study which is now reported.

Several other tests of the constancy or well-being of the preparation were carried out from time to time (Table I). (I) Great changes in hydrogen ion con-

TABLE I

*The results of analyses of samples of the blood perfusing the heart and lungs at the beginning and end of the period of observation in a number of experiments. The shortest interval between any pair of measurements was fifty minutes, the longest two hours and five minutes*

Dog number	At the beginning of the experiment				At the end of the experiment			
	O <sub>2</sub>	CO <sub>2</sub>	pH	Lactic acid	O <sub>2</sub>	CO <sub>2</sub>	pH	Lactic acid
	vol- umes per cent	vol- umes per cent		mgm. per 100 cc.	vol- umes per cent	vol- umes per cent		mgm. per 100 cc.
119	11.24	8.84			12.45	8.69		
120	12.34	8.36			12.25	8.10		
138	16.50	7.40			15.80	8.10		
147		9.76				11.02		
155	14.02	8.45			13.38	9.02		
164	13.35	7.70			12.94	8.10		
165	11.30	6.30			12.02	7.07		
182	15.91				14.79			
203	15.70	7.23	7.68	47.50	15.12	7.84	7.62	51.2
204				42.50				48.5
207			7.64	50.30			7.62	55.7
208			7.72	40.70			7.67	44.8
209	16.27	9.24	7.64	38.75	15.40	8.60	7.67	41.6

centration, as measured by the colorimetric method of Hastings and Sendroy (6) were not encountered. The high degree of alkalinity is, of course, due in part to the fact that only the heart and lungs instead of the whole

<sup>2</sup> A description of the stromuhr and the stopcock will be published elsewhere.



body were producing carbon dioxide, and in part to the large volume of the ventilation employed, but so large a volume was necessary in order to maintain the lungs in good condition and the blood sufficiently oxygenated. The effect of this set of artificial conditions is also reflected in the low content of carbon dioxide in the blood. During the period when measurement of metabolism was going on, there was but little change in concentration of either carbon dioxide or oxygen (Table I). (II) The presence and multiplication of bacteria in the perfusing blood was also investigated. The chief sources of infection lay in the rubber dam constituting the artificial resistance and in the rubber of the venous reservoir. But if these were kept, between experiments, in alcohol 70 per cent it was necessary to do no more to sterilize the apparatus than to wash the glassware and the rest of the rubber tubing with soap and water, to allow it to dry, and then, when the parts were assembled, to flush the whole with a few hundred cubic centimeters of fresh Ringer's solution just before the experiment. With the use of this procedure it was usually impossible to cultivate bacteria on blood agar plates. Occasionally at the end of 3 hours, three or four colonies per cubic centimeter were found, on one occasion eighteen. (III) The concentration of lactic acid in the blood was measured (7) from time to time at the beginning and end of the periods of observation. The largest increase was 6 mgm. in each 100 cc. of blood. (IV) Oxygen and carbon dioxide content of the blood

were also measured (4) at the beginning and end of a number of experiments. Significant changes were not observed.

The heart was weighed within 15 minutes of the termination of the experiment. After injection with a barium sulphate mixture x-ray photographs were made to study the arterial blood supply. This investigation will form the body of a separate communication. The fat around the base of the heart as well as the aorta and the pulmonary artery were next removed. Their weight was subtracted from the total weight of the heart. The consumption of oxygen<sup>3</sup> was calculated according to the wet weight per gram of auricle and ventricle.

Only those experiments in which the following criteria were fulfilled were considered suitable for analysis:

1. The preparation must have attained and held for at least half-an-hour a steady state in regard to cardiac output, arterial and venous pressures, and rate of consumption of oxygen.

2. The respiratory quotient must have remained within reasonable limits; greater than 0.80 and less than 1.05.

3. At least two pairs of estimations of the consumption of oxygen must have been obtained differing from each other by less than 7 per cent (the estimated error of measurement of oxygen was 3.5 per cent) so that

<sup>3</sup> Throughout the remainder of the paper *consumption of oxygen* is employed instead of the more exact phrase —consumption of oxygen per gram of heart per hour.

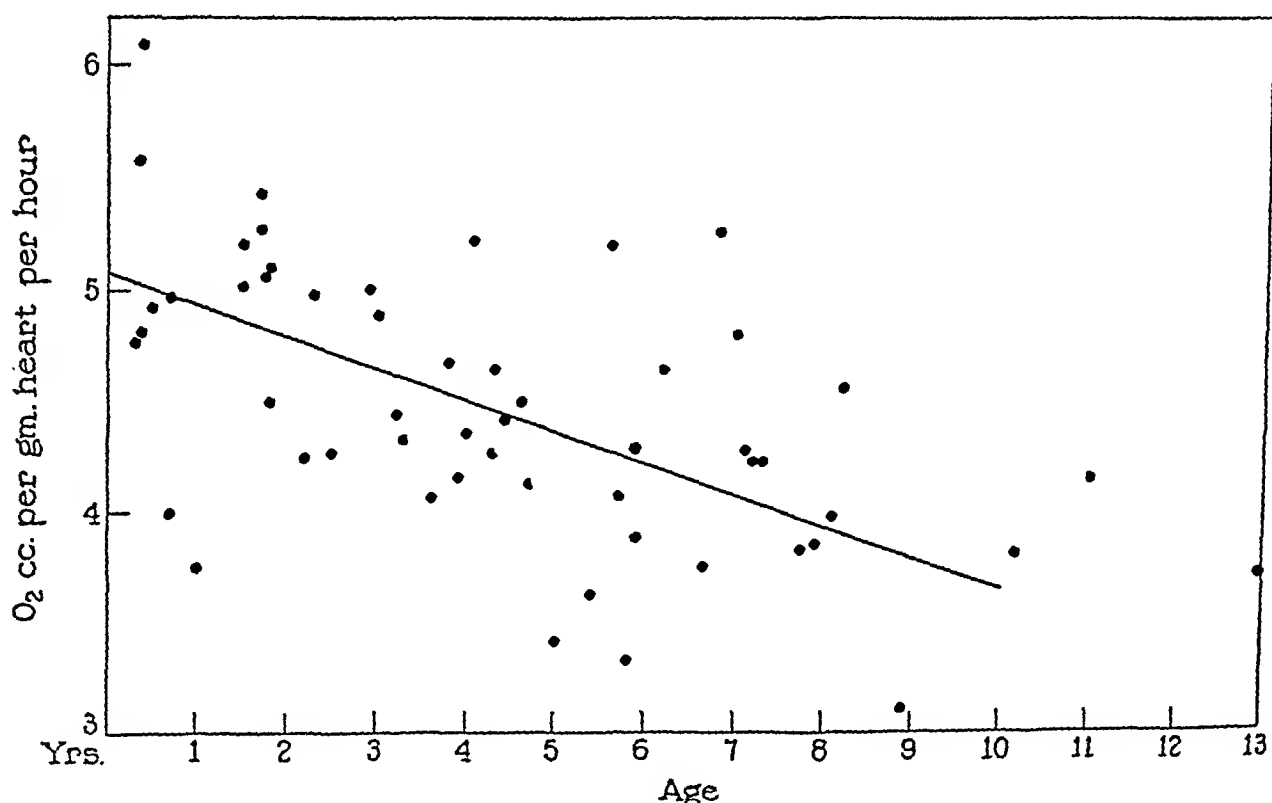


FIG. 2.

The values for the consumption of oxygen by the heart are plotted against the age of the animal and the regression line is drawn.

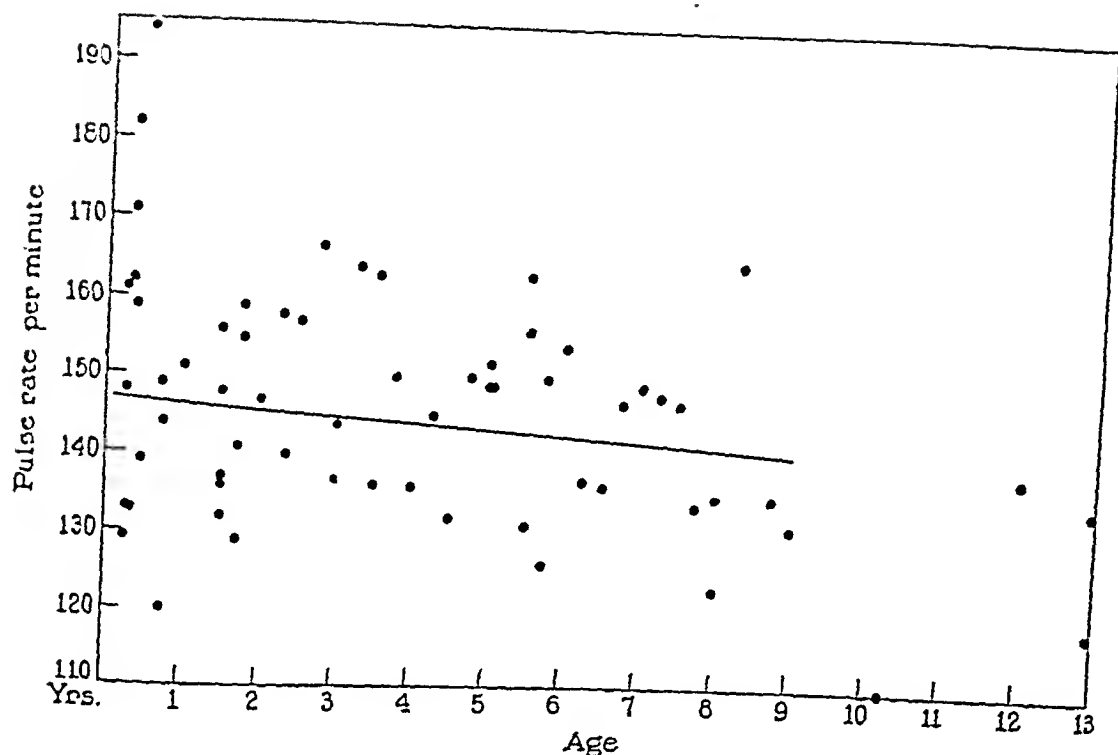


FIG. 3.

Observations on the rate of contraction assumed naturally by each heart are plotted in relation to the age of the animal and the regression line drawn.

there might be assurance that the system was in equilibrium.

There are, of course, in heart-lung preparations countless variable factors beside the two (age of the animal and consumption of oxygen by the heart) which were the subject of this study. Many are known and can either be regulated or their effect calculated, but many more are unknown. To the unknown ones is due the unaccountable variability of metabolism from one animal to the next. It is obviously advantageous that variation in the two factors under investigation be as great, and in all others as small, as possible. Attempt was made with reasonable success to eliminate variation in such factors as temperature, diastolic length of muscle fiber, cardiac output, venous and arterial pressure and composition of the blood in respect to concentration of oxygen, carbon dioxide, sugar, lactic acid and hydrogen ions. The frequency of the heart beat naturally varied. To regulate it was found to be impracticable. The size of the heart also varied due to the different ages of the animals. The influence of certain of these factors has been studied; their effect is described later.

#### RESULTS

The results are summarized briefly:

1. The consumption of oxygen per gram of

heart *decreases with age* (Figure 2). The crude coefficient of correlation was  $-0.6002$ .

2. The frequency of the heart beat *decreases with age* (Figure 3).

3. The consumption of oxygen per gram of heart *increases with rate* (Figure 4). The coefficient of correlation was  $+0.4706$ . The suggestion emerges accordingly that part of the decrease in the consumption of oxygen with age may be due to the decrease in the frequency of contraction. What influence frequency of contraction exerts on consumption of oxygen at successive ages can be calculated, were all the hearts assumed to beat at the same rate. The relation (frequency of contraction and consumption of oxygen) is shown in the curve (Figure 4) and is expressed by the formula:

Consumption of oxygen per gram of heart per hour  $\approx 0.187$  number of contractions per minute  $+ 2.23$  (8).

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body were producing carbon dioxide, and in part to the large volume of the ventilation employed, but so large a volume was necessary in order to maintain the lungs in good condition and the blood sufficiently oxygenated. The effect of this set of artificial conditions is also reflected in the low content of carbon dioxide in the blood. During the period when measurement of metabolism was going on, there was but little change in concentration of either carbon dioxide or oxygen (Table I). (II) The presence and multiplication of bacteria in the perfusing blood was also investigated. The chief sources of infection lay in the rubber dam constituting the artificial resistance and in the rubber of the venous reservoir. But if these were kept, between experiments, in alcohol 70 per cent it was necessary to do no more to sterilize the apparatus than to wash the glassware and the rest of the rubber tubing with soap and water, to allow it to dry, and then, when the parts were assembled, to flush the whole with a few hundred cubic centimeters of fresh Ringer's solution just before the experiment. With the use of this procedure it was usually impossible to cultivate bacteria on blood agar plates. Occasionally at the end of 3 hours, three or four colonies per cubic centimeter were found, on one occasion eighteen. (III) The concentration of lactic acid in the blood was measured (7) from time to time at the beginning and end of the periods of observation. The largest increase was 6 mgm. in each 100 cc. of blood. (IV) Oxygen and carbon dioxide content of the blood

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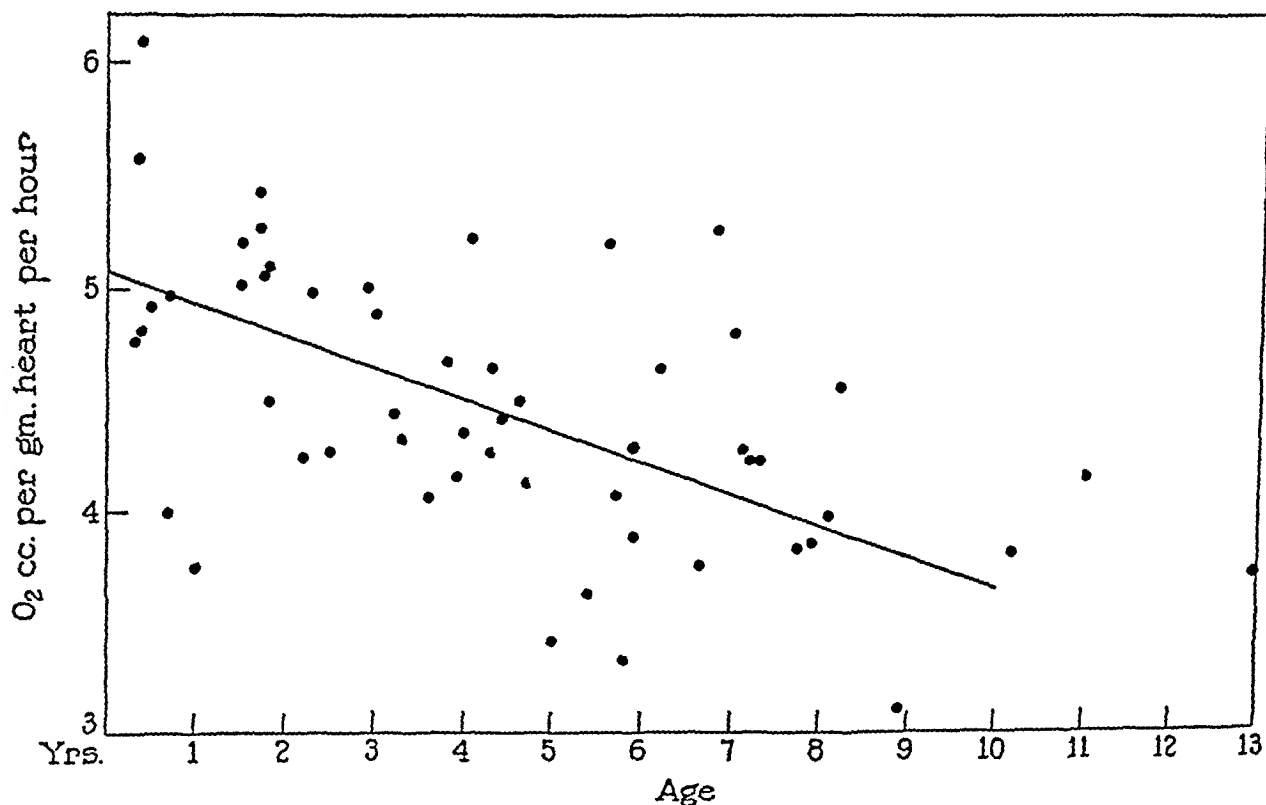


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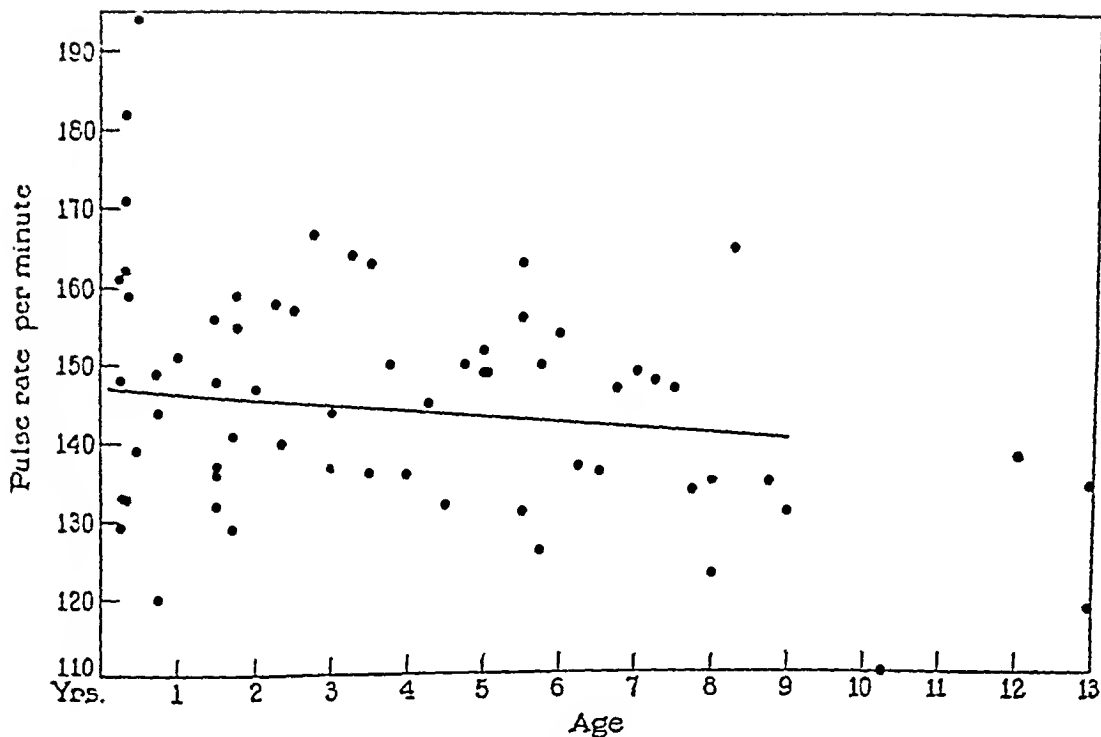


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The influence of this relation with respect to age can now be taken into account by modifying the

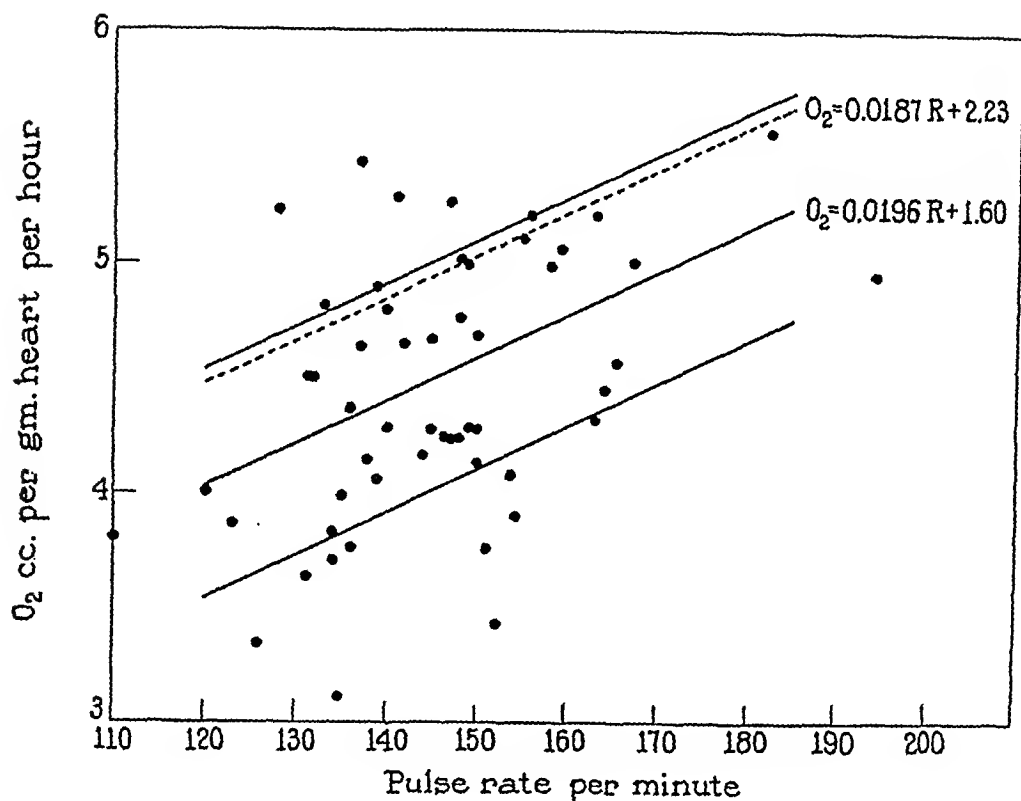


FIG. 4.

The rate of consumption of oxygen of each heart is plotted according to the frequency of contraction. The regression line is drawn in. The dotted line represents the equation expressing the relation between frequency of contraction and consumption of oxygen obtained in a different manner (8).

original results (Figure 2). The method of partial correlation (9) permits this calculation. The resulting coefficient of correlation is found to be  $-0.5016$ . This figure, though less than the original one ( $-0.6002$ ) is still significant in that the probability of the occurrence of this relation by chance is only one in a thousand.

4. The size of the heart is related both to age and to the amount of oxygen consumed. Larger hearts use less oxygen per unit of weight than smaller ones (Figure 5), the coefficient of correlation being  $-0.5421$ . Since adult hearts are larger than younger ones (Figure 6) a coefficient of correlation of  $+0.5852$  of age with size results. It is obvious that as the heart increases in size with age (Figure 6), and since the rate of consumption of oxygen per unit of weight decreases with increase in the weight of the heart (Figure 5), the apparent decrease in consumption of oxygen with age may, in part, be due to increase in size of the heart. Size of the heart and frequency of its beat both accordingly affect the consumption of oxygen per unit of weight apart

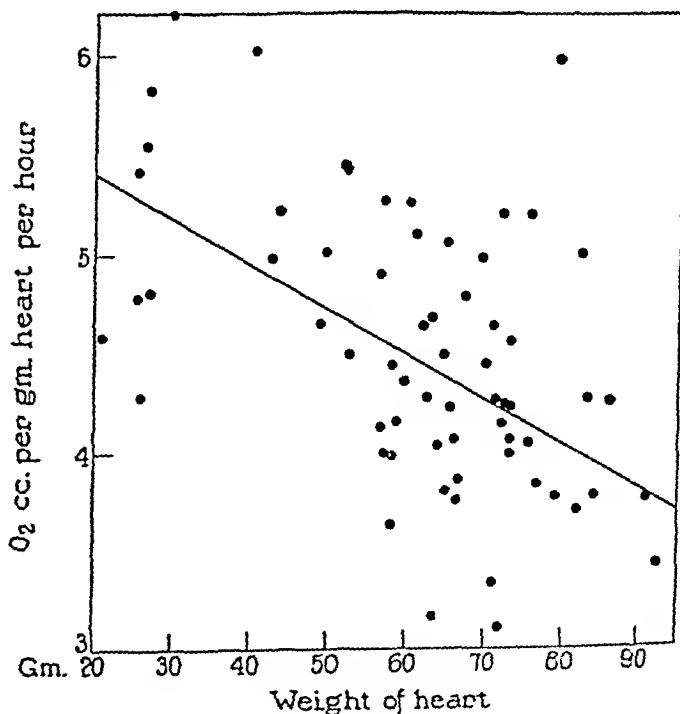


FIG. 5.

This diagram expresses the relation found between size of heart and consumption of oxygen per unit of weight. Dots indicate the individual observations; the line, the regression equation.

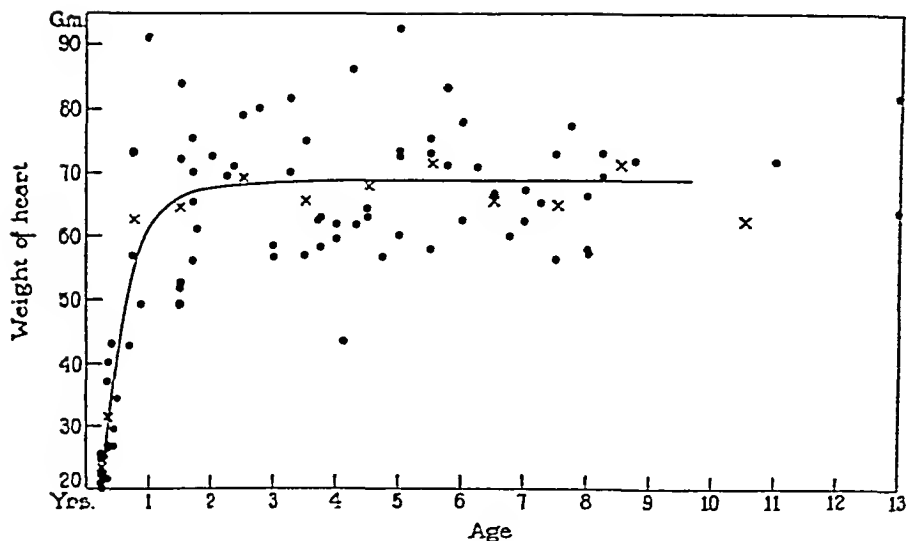


FIG. 6.

Weights of hearts are charted as dots according to age. The curve was drawn in the manner of Brody (*Growth and development. III. Growth rates, their evaluation and significance. Missouri Agric. Exper. Station. Research Bull. 97, January 1927*).

from the effect of age. The effect of age alone therefore (on the consumption of oxygen) dwindles; the partial correlation coefficient expressive of this fact (the relation between age and consumption of oxygen) falls to  $-0.3561$ . This figure is of doubtful significance, for the probability of its expressing an accidental relationship is greater than one in twenty.

But the curve of increase in size of heart with age is clearly not linear but logarithmic (Figure 6). After  $1\frac{1}{2}$  years of age, little if any change in size of the heart occurs and exerts, consequently, little influence on the relation between oxygen and age. The coefficient of correlation between weight and age, for dogs above one year old, drops to an insignificant figure ( $+0.2172$ ), while that between weight and consumption of oxygen increases ( $-0.5904$ ). If the process of calculating the partial correlation coefficient of age and oxygen, independent of the influences of weight of heart and frequency of contraction is repeated for the groups above one year of age, the coefficient is somewhat enhanced, rather than reduced, as it was in the whole group, by making allowances for the two additional variables. The coefficient is now  $-0.6040$  which is, according to Fisher (10), a highly significant figure.

If corrections are made for the functions of size and frequency of contraction, a significant degree of correlation between age and consumption of oxygen remains. Without these corrections a high degree of correlation exists and is expressive of the integrated behavior of the heart as a whole according to age, for those changes in frequency and size which have been discounted are themselves likely to be intrinsically connected with changes in function with time both in the heart and in the whole animal.

5. The amount of work to be performed by the hearts could be regulated, as is customary in these preparations, by arranging venous and arterial pressures. They were, in fact, so arranged that the correlation between age and work turned out to be insignificant, the coefficient being  $+0.0918$ . In this manner work, although directly related to consumption of oxygen, was shown to exert no greater influence on one age group than on another.

6. When all the variables of which one can take account are considered, and their influence upon the consumption of oxygen is weighed in their relation to age, it appears that consumption of oxygen by the heart decreases as that organ grows older. A considerable and unaccountable

variation still exists which, expressed in terms of the standard error of estimate, amounts to  $\pm 0.5$  cc. of oxygen per gram of heart. Its occurrence may be taken as a simple and clear statement of the fact that additional variable quantities are operative of which no knowledge exists.

These results suggest that decrease in frequency of beat and increase in size of the heart with age do not account altogether for the decrease with age which has been found in the rate of consumption of oxygen by that organ. Although in the intact animal all of these factors are operative, perhaps even to a greater degree than in the heart-lung preparation, there occurs in addition some change expressive of a more fundamental one in the heart with age. Whether the change involves substitution of muscle by some less active tissue, fat for example, or some intrinsic change or changes in composition of the muscle fibers, is a question which remains unanswered. Evidence that changes of both sorts occur, exists, but to find it has naturally not formed part of this investigation.

#### CONCLUSION

Decrease in the consumption of oxygen with age was observed in heart-lung preparations made in pure-bred female wire-haired fox terriers of known age living under similar environmental conditions and having been given similar food. Those variable factors in the life of the preparation which could be arranged were controlled; the effects of those which were uncontrollable were calculated. The degree of relationship between age and consumption of oxygen per unit of weight of heart was considerably less when account was taken of certain functions found to be related to both (age and oxygen consumption) but was still significant.

The evidence suggests that, although rate of contraction, size, and, in intact animals, the amount of work done, play a part in the decrease

of metabolism with age, there is in addition a change in structure of the heart or in its composition with age which leads to reduction in the amount of oxygen consumed by each unit of its weight.

A technical observation is believed to be of sufficient importance to deserve mention in the conclusion. When the lungs are exposed to ether even for brief periods, they are likely to become edematous—but apparently not otherwise.

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# STUDIES OF SODIUM AND POTASSIUM METABOLISM. THE EFFECT OF POTASSIUM ON THE SODIUM AND WATER BALANCES IN NORMAL SUBJECTS AND PATIENTS WITH BRIGHT'S DISEASE

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The work presented here was undertaken primarily for the purpose of ascertaining the effect of the ingestion of moderate amounts of potassium on the sodium balance of normal individuals and patients with Bright's disease. It was felt that such information would be of particular significance in appraising the efficiency of potassium salts as diuretics in the treatment of nephritic edema. Potassium chloride was selected as the means of administering the potassium in order to avoid the alkaline or cathartic effects of other potassium salts.

Incidentally, the experiments in supplying complete data on sodium and potassium balances and weight changes provide an opportunity for correlating sodium and potassium balances with the water balance of the body.

## LITERATURE

In 1873 Bunge (1) in well controlled experiments showed that the ingestion of 387 milliequivalents of potassium as phosphate, citrate, or chloride (which as KCl is 28.2 grams) during a single day resulted in an increase in urinary sodium and chloride. From this he concluded that the ingestion of potassium salts caused withdrawal of sodium and chloride from the body. He left unanswered the question whether the continued intake of potassium would cause a continued withdrawal, especially under the condition of low sodium intake. In 1896 Pugliese (2) attempted to answer this very question by experiments on dogs. Some experiments showed an augmentation of sodium excretion after the ingestion of a potassium salt, while others failed to do so. Though his results were inconclusive he felt that potassium chloride acted as a diuretic. In 1911 Hart, McCollum, Steenbock and Humphrey (3) concluded that the amounts of potassium in the feed of herbivora did not affect their retention of sodium and chloride or influence their sodium chloride consumption.

Subsequent work has added to this conflicting evidence. Meyer and Cohn (4), Gerard (5, 6, 7), and Wiley, Wiley and Waller (8) found that potassium administration to normal human subjects or animals caused a negative sodium balance. Miller's (9) experiments provided conflicting evidence. Loeb et al. (10) observed that potas-

sium chloride administration in a normal subject caused a slight increase in total base excretion, probably due to increased urinary sodium, but no definite diuretic effect. They felt the observed changes in excretion were the result of changes in the urinary pH which resulted from the difference in the rate of excretion of potassium and chloride, and that the observed changes were not produced by a specific potassium ion effect. On the other hand, Richards, Godden and Husband (11) and Bassett, Elden and McCann (12) found that the addition of potassium citrate to the diet of a normal subject did not cause a negative sodium balance but did have a diuretic action.

In experiments on a nephritic patient Loeb et al. (10) obtained results similar to those observed in their normal subject. However, Bassett, Elden and McCann (12) found in contradistinction to their results on a normal subject that large doses of potassium citrate to a nephritic patient caused a diuresis and negative sodium and chloride balances.

Many qualitative studies have been reported on the differential effect of sodium and potassium ingestion on water balance. Blum (13, 14) in 1909 and 1920, in studying the edemas of diabetics, observed that the giving of  $\text{NaHCO}_3$  caused increase in weight, suppression of urine, and diminished chloride excretion, while the giving of  $\text{KHCO}_3$  had exactly the opposite effect. Meyer and Cohn (4) showed the same striking contrasts in infants following sodium and potassium ingestion where there was no limitation to the fluid intake. Schloss (15), Veil (16), Labbe and Voille (17), and Kempmann and Menschel (18) have reported similar findings.

The work of Gamble, Ross, and Tisdall (19) demonstrated the approximate constancy of the osmotic concentrations of the body fluids, and established a relationship between base and water balances. This led them to differentiate between the loss of extracellular water and intracellular water by the sodium, potassium and nitrogen content of the urine. Peters and Van Slyke (20) have summarized the findings of Gamble and coworkers by the following two formulae:

$$\frac{\text{Na} - 0.425\text{K}}{148} = \text{liters of extracellular water lost,}$$

$$\frac{\text{K} - 0.017\text{Na}}{112} = \text{liters of cellular water lost,}$$

where Na and K represent milliequivalents excreted in excess of intake. Blumgart and his collaborators (21),



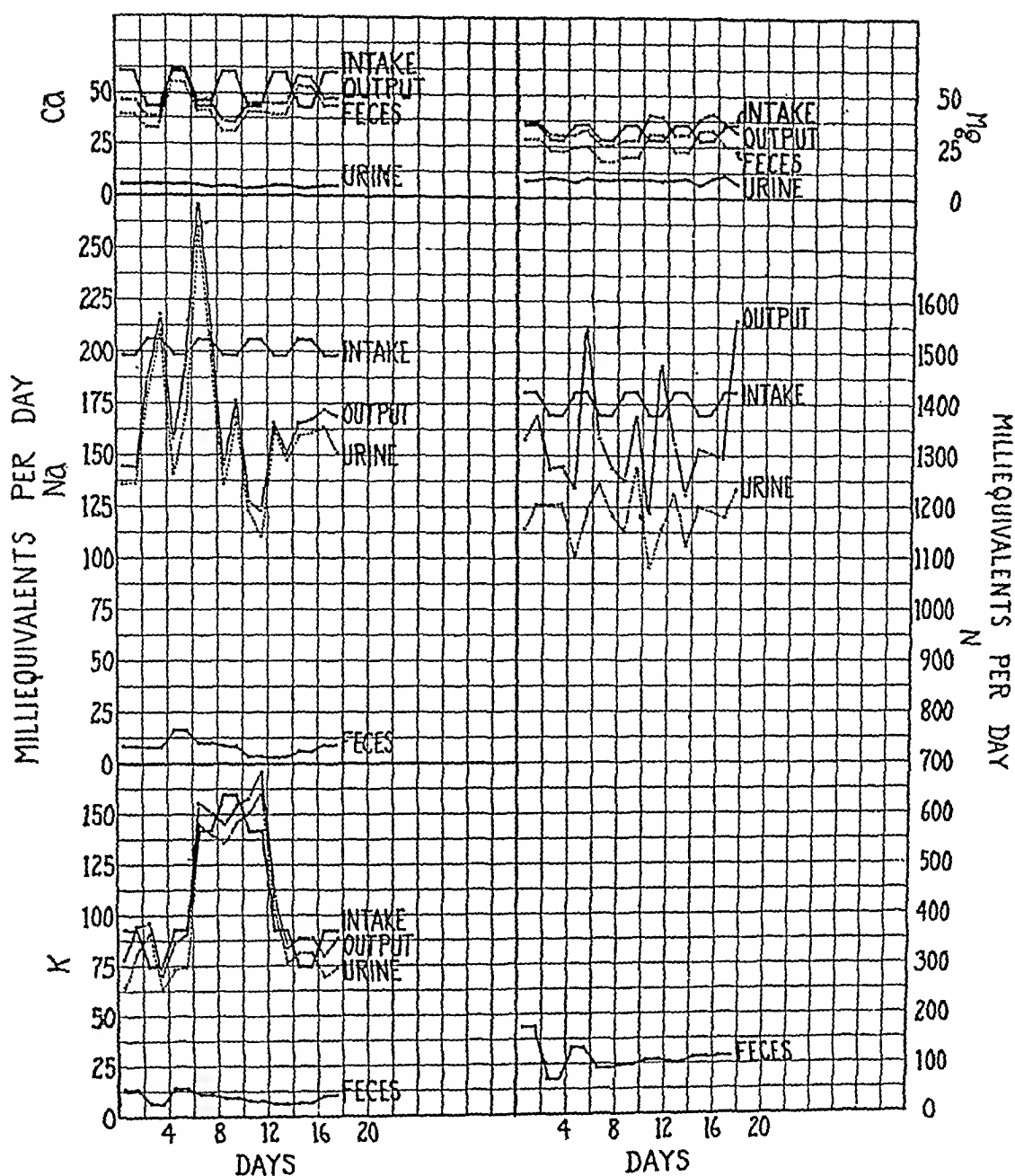


FIG. 1. DAILY BALANCES, EXPERIMENT I, NORMAL SUBJECT "B"

using this method of differentiating extracellular from intracellular water, have shown that approximately ninety per cent of the fluid lost from the body following the administration of mercurial and xanthine diuretics represents a loss of extracellular body fluids.

Recently Lavietes, D'Esopo and Harrison (22) have called attention to the discrepancies caused by fluctuations in the base concentration of body fluids in water balance calculated from base balances. They also showed that over short intervals of time estimated water balances from sodium or chloride balances agreed closely with the actual changes in weight in normal and nephritic subjects. Their data thus indicate that such weight changes over short intervals of time represent loss or retention of extracellular fluid and confirm the findings of the investigators already mentioned (10, 12, 21).

Though the more recent papers referred to above (10,

11, 12, 21, 22) have appeared since our experiments were completed, our results are reported briefly because the slightly different experimental conditions provide additional information in a field of investigation where quantitative data are accumulated only at the expense of much time and labor.

#### OUTLINE OF EXPERIMENT

Two healthy young adult males, Subjects M and B, served as normal subjects. Both were up and about carrying on their usual ward and laboratory routine during the course of the experiment.<sup>1</sup> Each subject was

<sup>1</sup> Subject "B" contracted a severe cold on the thirteenth day of Experiment I. The last six days of this experiment, therefore, cannot be considered as on a normal subject.

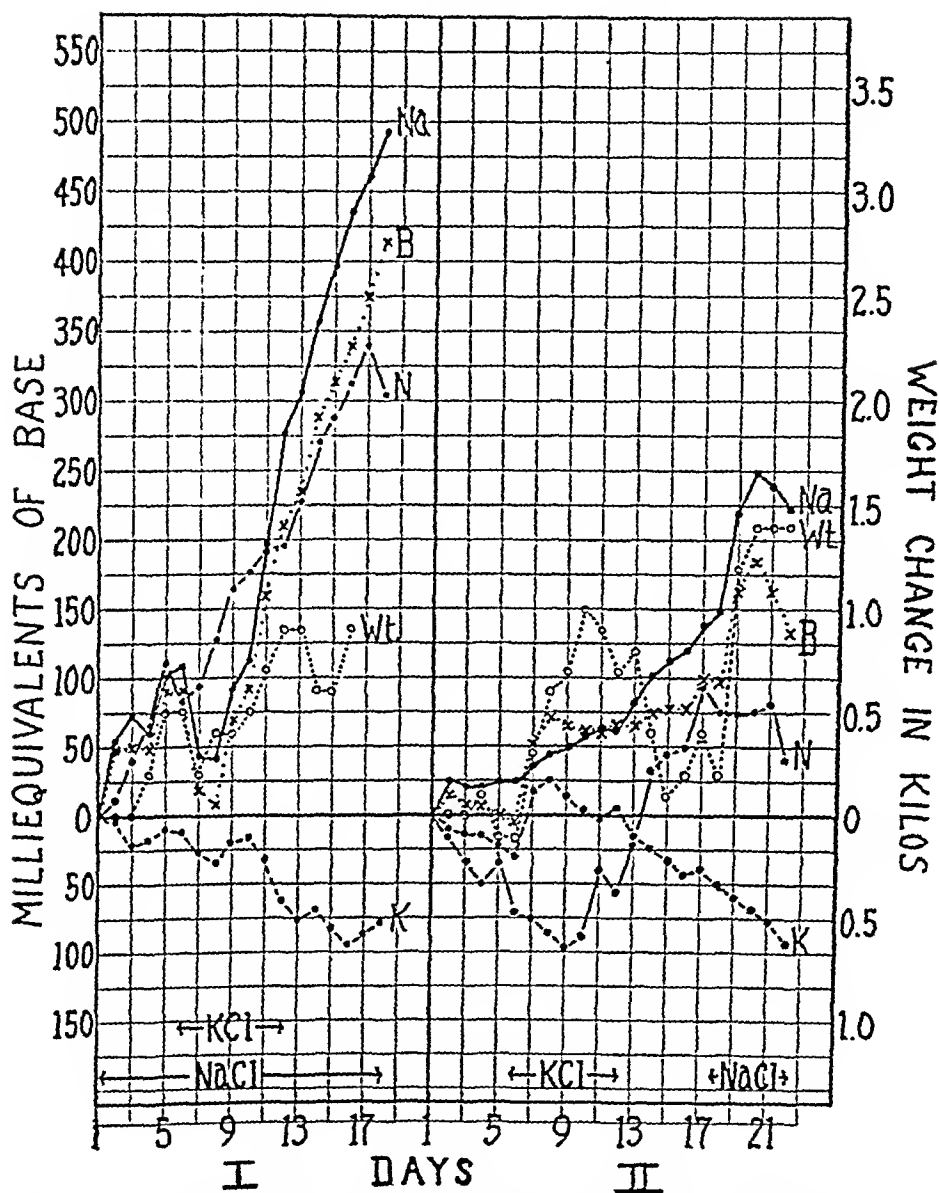


FIG. 2. DAILY CUMULATIVE RETENTIONS, EXPERIMENTS I AND II WITH NORMAL SUBJECTS  
 Meq. of base  $\times 4 =$  meq. of nitrogen.

given a constant weighed and analyzed diet for a fore-period of six days and throughout the experiment. The diet consisted in each case of two different daily diets which were alternated during the experimental period. Each diet was given for two consecutive days followed by the other diet for the next two days and so on, alternating every two days.<sup>2</sup> The meals were prepared and served with as great accuracy as is possible in handling foodstuffs. Water was allowed as desired, the volume taken being recorded. At three different times during the

<sup>2</sup> In Experiment III the two different daily diets were given on alternate days.

experiments a duplicate of each of the diets was prepared. The three meals for the day reached the laboratory on dishes similar to those used for serving, and the food was removed from them with the same implements. Three duplicates of each diet were analyzed and gave the mineral intake data for the experiments. Our method of preparing the diets for analysis has been described (23).

The twenty-four hour urine specimens were collected without any preservative, the specimens being kept in an ice-box until the end of the period. The pH, ammonia, and titratable acidity were determined daily.

Stool specimens were collected over 48-hour periods.

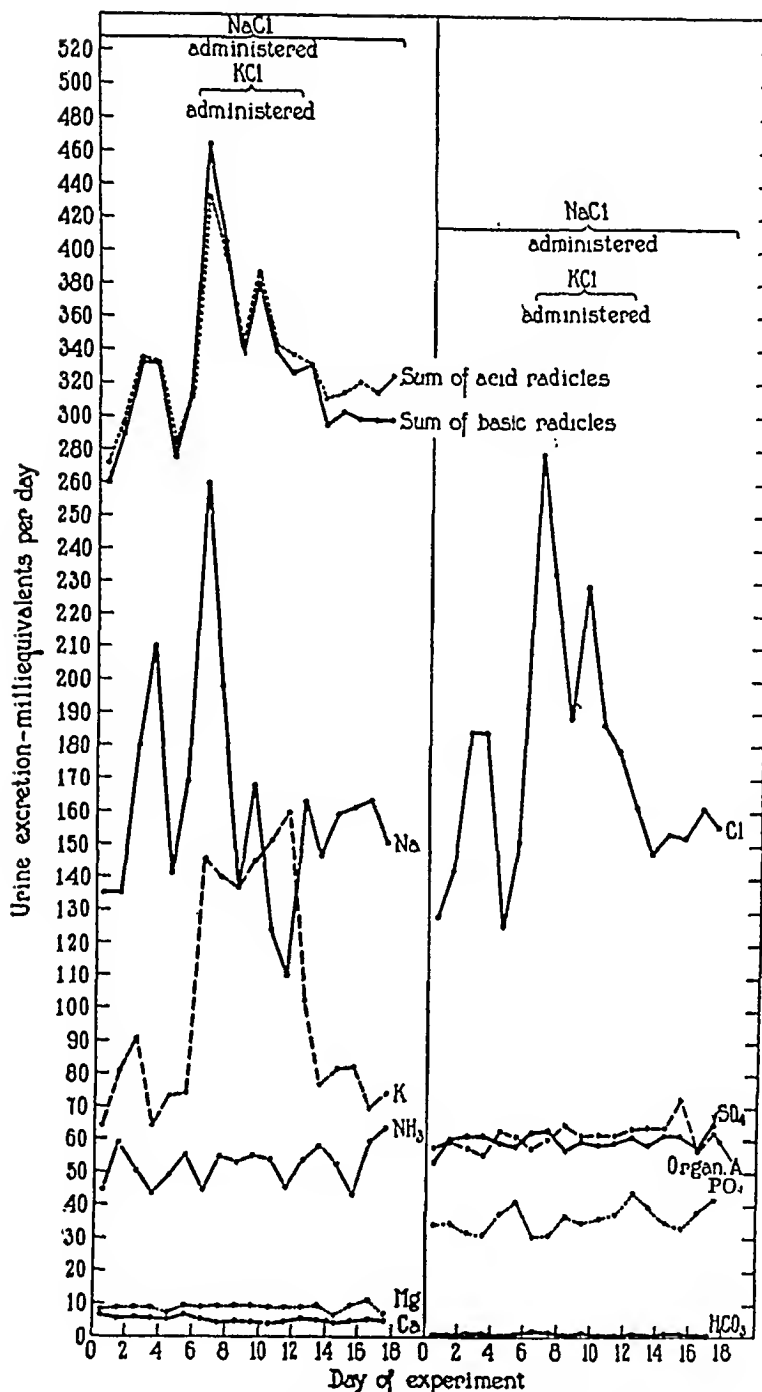


FIG. 3. URINARY EXCRETION OF BASIC AND ACID RADICLES. EXPERIMENT I

These were marked off with carmine. During the first two days of the experiment carmine (0.2 gram) was taken with every meal, thus coloring the entire stool specimen of the period. This was repeated during each alternate 48-hour period. The preparation of the stool specimens for analysis has been described (23).

The rate of urinary excretion of each of the acid and basic radicles and of nitrogen was likewise determined before, during, and after periods in which potassium chloride was administered.

The analytical methods are described in Peters and Van Slyke (23) as follows: for urine pH, page 792; titratable acidity, p. 828; ammonia, p. 547; chloride, p. 833;

inorganic sulfate, p. 893; inorganic phosphate, p. 858; calcium, p. 425; magnesium, p. 779 and sodium and potassium, p. 727. For diets and feces the analytical methods are also given in Peters and Van Slyke; calcium, p. 766; magnesium, p. 784 and sodium and potassium, p. 730. All nitrogen determinations were carried out by the usual Arnold-Gunning Kjeldahl procedure.

#### RESULTS

The daily balance data are presented graphically. The ordinates of the *balance* graphs, Figures 1, 4, 6, and 9, represent milliequivalents of the base

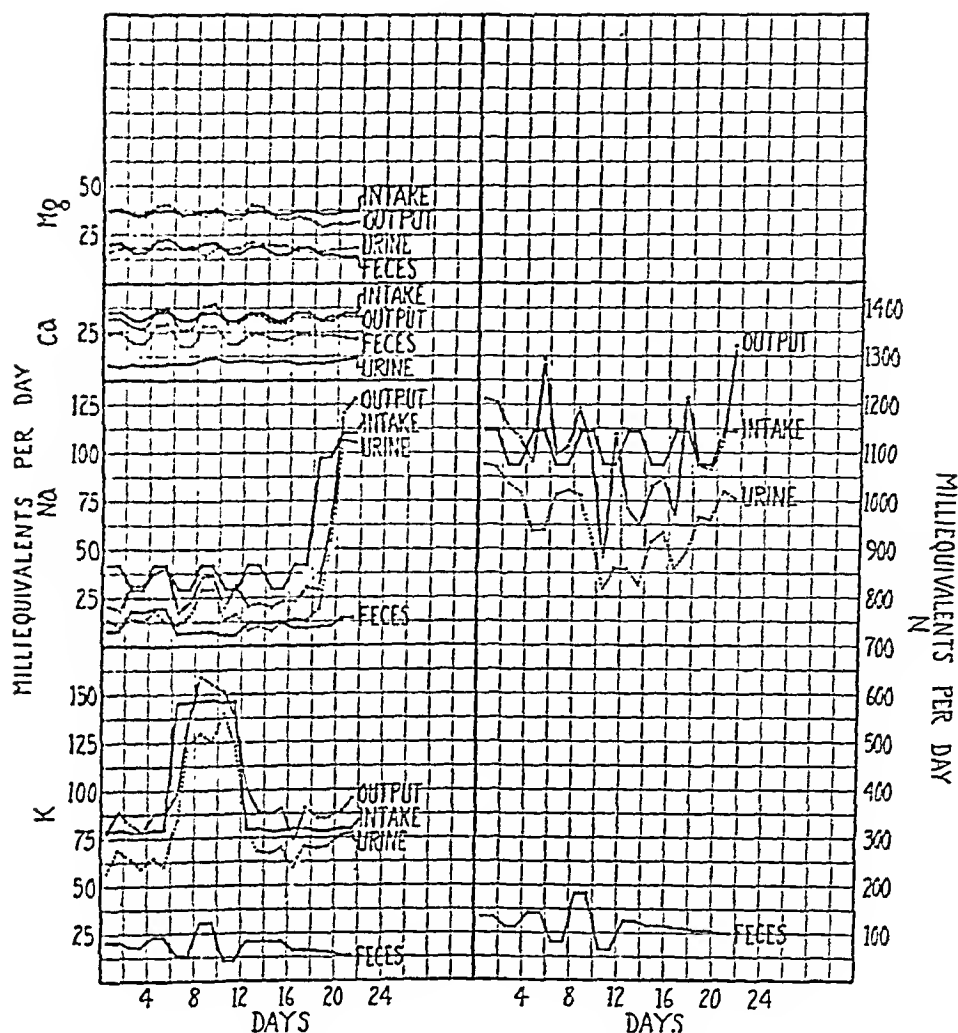


FIG. 4. DAILY BALANCES, EXPERIMENT II, NORMAL SUBJECT "M"

and nitrogen ingested or excreted each day of the experiment. Curves are given showing the daily intake and total output. Also curves showing the division of the total output between urinary and fecal excretions are given. On days that blood samples were withdrawn, the difference between the total output and the sum of urinary and fecal excretions represents the loss of material from the body in the blood sample removed. Examination of the intake curve of each element will show the nature of the diet as regards it, how much of each element was included in the basal diet, and the periods over which added salts were given, together with the amount of base added. All added sodium and potassium were given in the form of chlorides.

The *retention* graphs, Figures 2 and 7, present the cumulative retention of sodium, potassium, and nitrogen compared to the weight change. The ordinate scales for weight and base retention, respectively, are so arranged as to make 150 m.eq. of base correspond with a weight change of one kilo. In order to plot nitrogen retention values conveniently on the same graph a different scale for nitrogen and base had to be used. The note at the bottom of each graph indicates the multiple of the base scale that gives milliequivalents of nitrogen. The sums of the sodium and potassium retentions are also plotted and are designated on the graphs by the letter B.

The ordinates of the graphs depicting the urinary excretion of acid and basic radicles, Figures

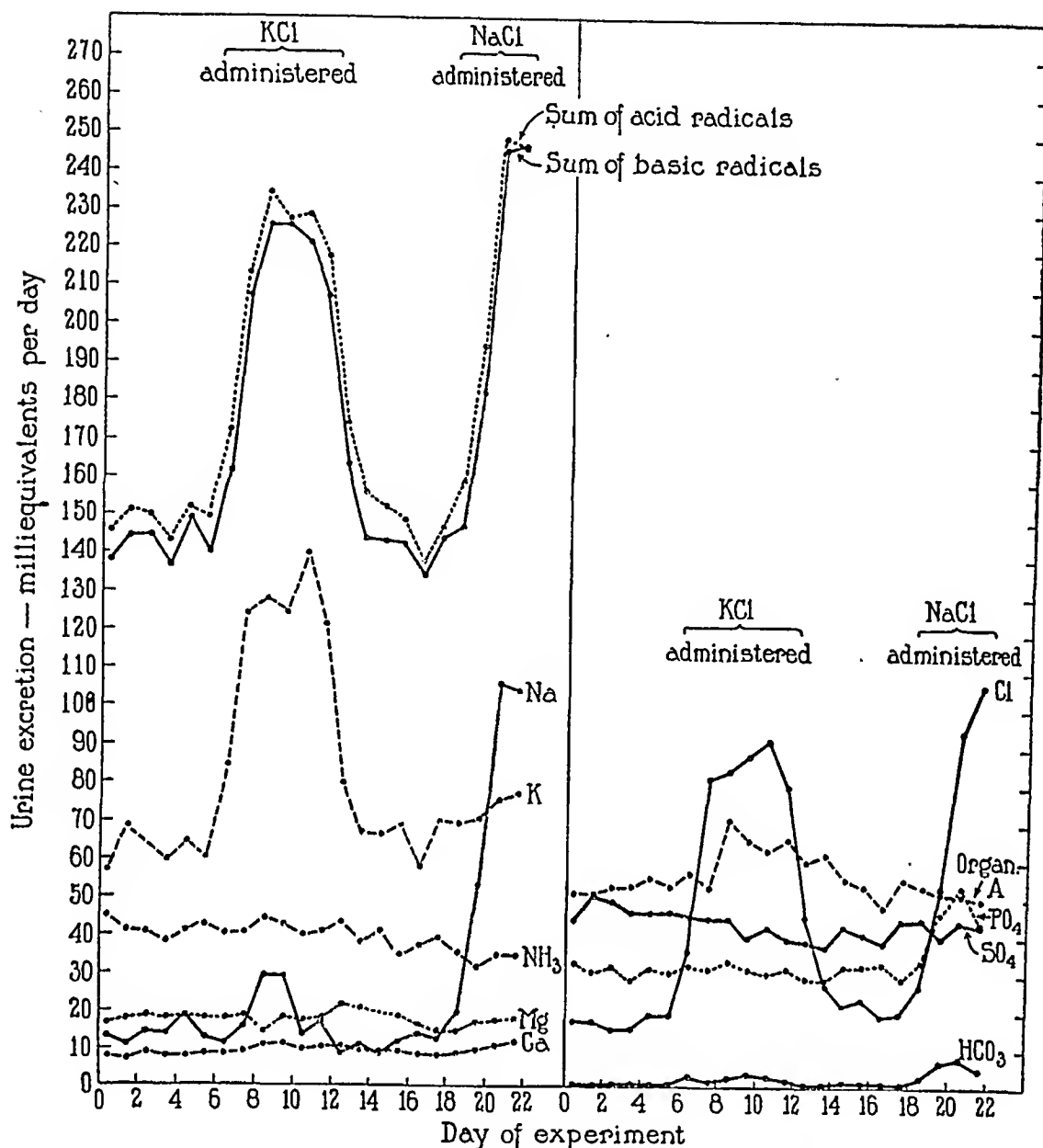


FIG. 5. URINARY EXCRETION OF BASIC AND ACID RADICLES, EXPERIMENT II

3, 5, 8, 10, represent milliequivalents of individual and total cations and anions in 24-hour urine specimens. The sum of the total cations and sum of the total anions of the urine have been plotted in order to compare the positive and negative equivalents.

The balances of the acid radicles could not be included in the metabolism graphs as our methods of analyses of feces and food interfered with the determination of many such radicles. Furthermore, the metabolic formation of acids (e.g. of sulphuric and phosphoric acids from organic compounds of S and P) renders difficult any attempt to balance total anion intake and output.

*Experiment I. Normal Subject B. Weight 68 kgm. (Figures 1, 2, 3. Tables I, II.)*

The subject during a six-day fore-period and eighteen-day experimental period was on a diet moderately high in sodium. The daily basal diet contained an average of 202 m.eq. of sodium (equivalent to 11.7 grams of NaCl). Of this total amount of sodium 68.4 m.eq. per day were derived from the addition of 4 grams of sodium chloride to the food. During Period II, 67 m.eq. of potassium, as 5 grams of potassium chloride, were added to the basal diet of Periods I and III.<sup>2</sup>

Table I summarizes the balance data per period.

Table II summarizes per period the per cent of intake

<sup>2</sup> See footnote 1, p. 924.

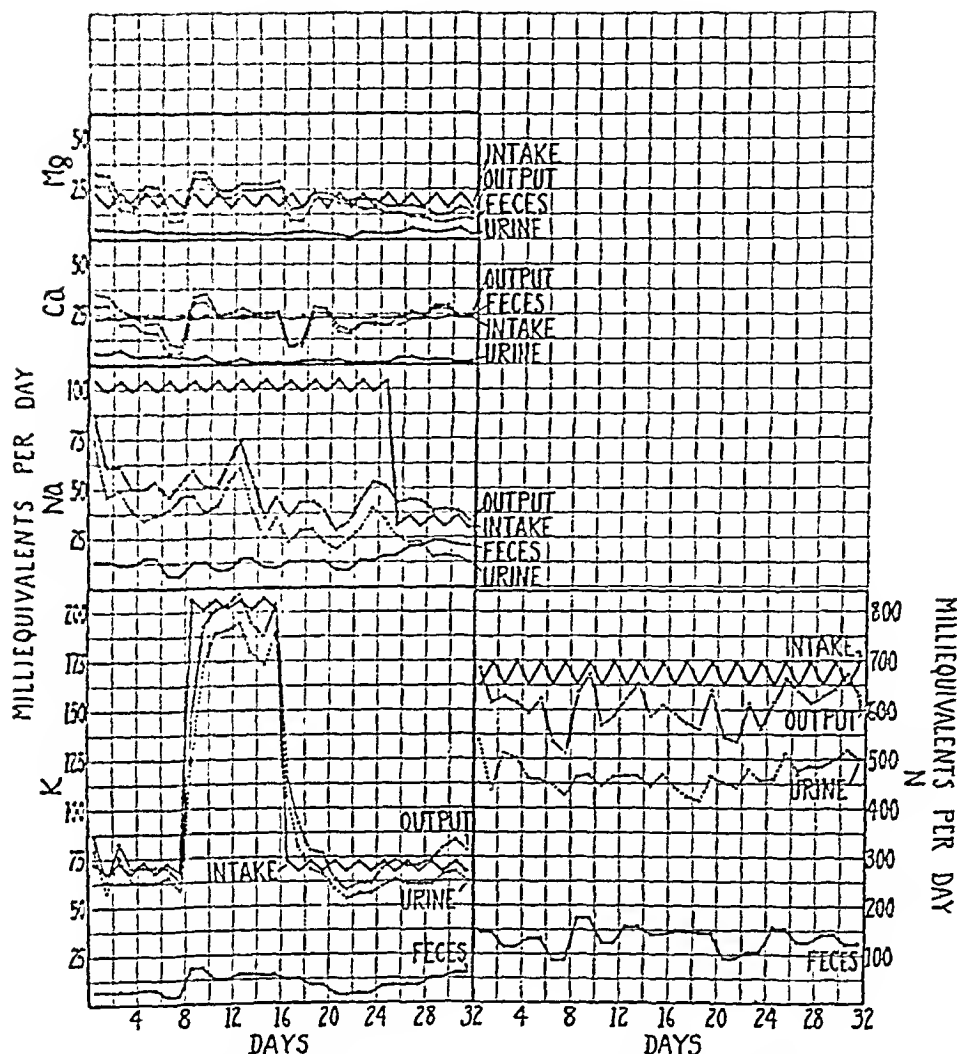


FIG. 6. DAILY BALANCES, EXPERIMENT III, NEPHROTIC PATIENT

excreted in the urine and feces<sup>4</sup> and the daily fecal excretion.

*Experiment II. Normal Subject M. Weight 70 kgm. (Figures 2, 4, 5. Tables III, IV.)*

The subject was on a moderately low sodium diet of 29 to 42 m.eq. of sodium per day, equivalent to 1.68 to 2.43 grams of NaCl. During Period II, 67 m.eq. of potassium, as 5 grams of KCl, were added per day to the basal diet of Periods I and III. In Period IV, 68.4 m.eq. of sodium, as 4 grams of NaCl, were added per day to the basal diet.

<sup>4</sup> We wish to call attention to the fact that the fecal excretion does not necessarily represent the unabsorbed portion of the ingested substance.

*Experiment III. Subject K, with nephrosis.<sup>5</sup> (Figures 6, 7, 8. Tables V, VI, VII.)*

The subject, previous to the experiment, had been on a low salt diet to combat edema. He was nineteen years of age and when edema-free weighed approximately 59 kilos. He had at this time a urea clearance of 42 per cent of normal and a blood pressure of 130/80 mm. Hg. His serum proteins were 4.7 grams per cent, the albumin being 1.6 gram per cent. During an eight-day foreperiod and the first three experimental periods of eight days each, the patient took 68.4 m.eq. (4 grams) of sodium chloride per day in addition to the 332 m.eq. of sodium in the diet, making the total daily sodium intake 102 m.eq. (equivalent to 5.9 grams of NaCl). In Period

<sup>5</sup> A summary of the case history has been given by Van Slyke et al. (see Case 65 (24)).

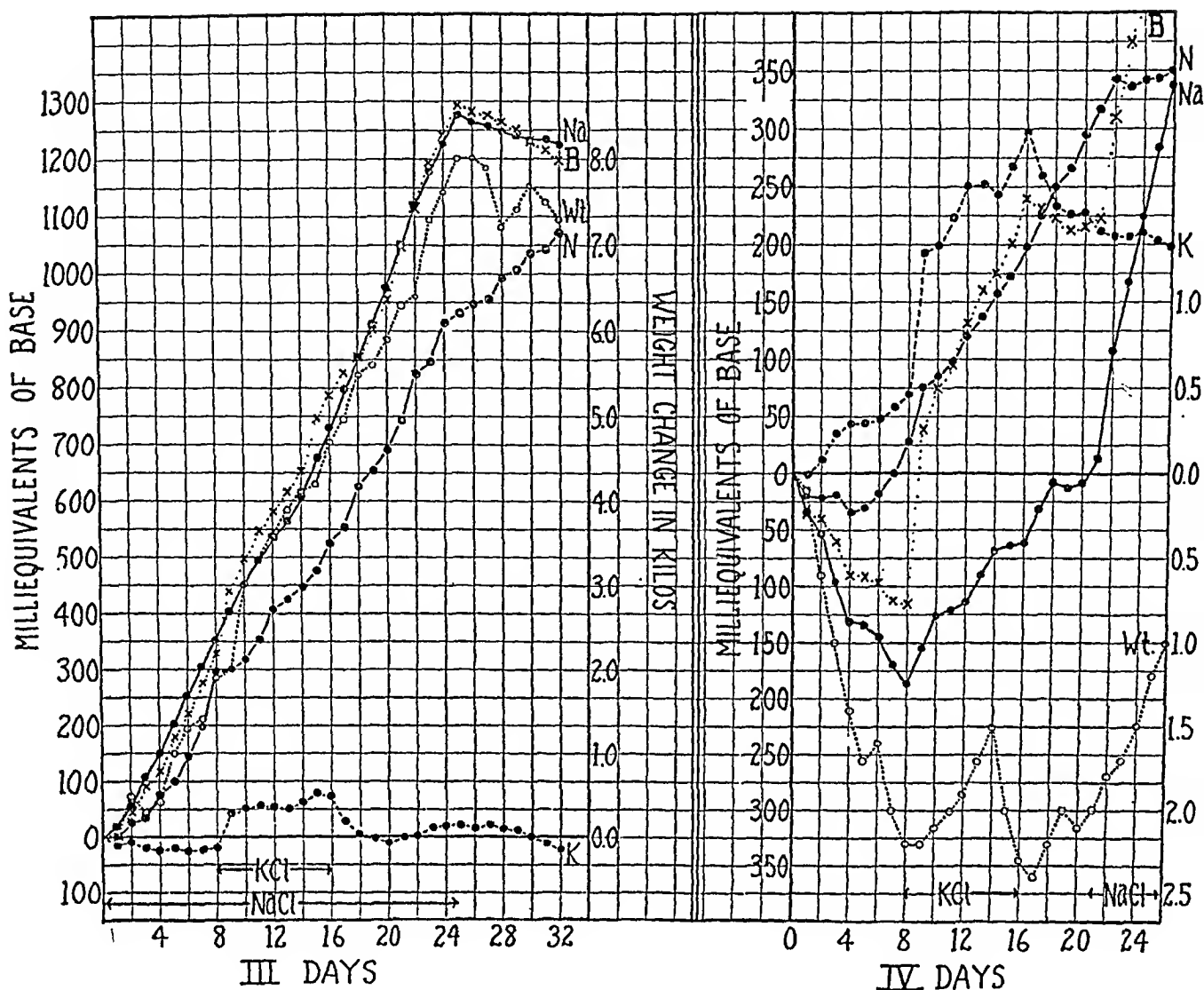


FIG. 7. DAILY CUMULATIVE RETENTIONS, EXPERIMENTS III AND IV

Experiment III, m.eq. base  $\times 2 =$  m.eq. of nitrogen.Experiment IV, m.eq. base  $\times 4 =$  m.eq. of nitrogen.

II, 134 m.eq. (10 grams) of potassium chloride were added to the diet and salt of Period I. Period III was similar to Period I. During the fourth period the basal diet without the added sodium chloride was taken.

Table VII shows the changes in the edema of this patient during the experimental period.

*Experiment IV. Subject L, with terminal chronic hemorrhagic nephritis.*<sup>6</sup> (Figures 9, 7, 10. Tables VIII, IX.)

The subject was 30 years of age, and weighed 57 kilos. His urea clearance was reduced to 12 per cent of normal. His serum proteins were 5.0 grams per cent, the albumin being 2.5 grams per cent. He had moderate edema of his ankles. His blood pressure was 150/95.

During Period I the patient was given a low sodium diet averaging 28 m.eq. of sodium or the sodium equiv-

alent of 1.6 gram of NaCl per day. During Period II KCl was added to the basic diet. On the first day 10 m.eq. as 10 grams of KCl were added. This amount resulted in such gastro-intestinal distress that the dose was reduced by half throughout the rest of the period. Period III was the same as Period I. In Period IV 4 m.eq. of sodium, as 4 grams of NaCl, were added to the basal diet.

#### DISCUSSION

##### 1. Excretion of the added potassium chlori-

In Experiment I (a normal subject on a low salt intake), the potassium from the 5 gram potassium chloride ingested per day during Period II was excreted promptly. A negative potassium balance occurred both on the first day of potassium chloride ingestion (Figure 1) and during the entire potassium chloride period (Table I).

<sup>6</sup> A summary of the case history has been given by Van Slyke et al. (see Case 29 (24)).

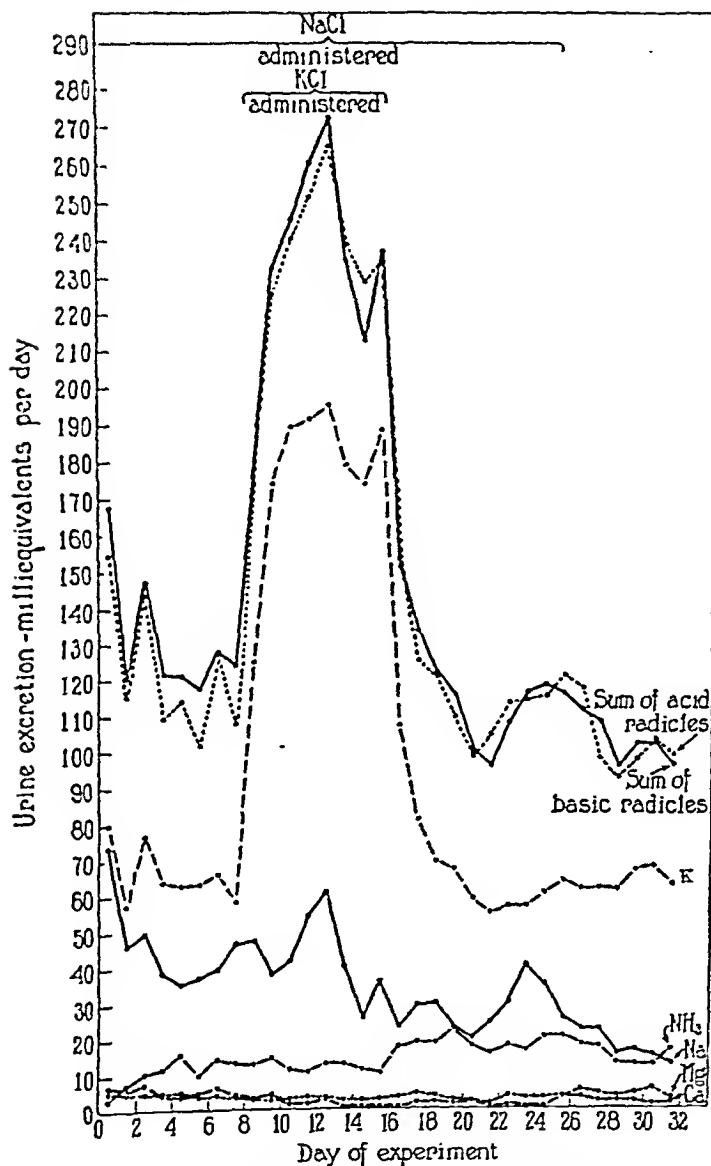


FIG. 8-a. URINARY EXCRETION OF BASIC RADICLES AND SUM OF ACID AND BASIC RADICLES, EXPERIMENT III

In Experiment II (a normal subject on a limited salt intake), the added potassium was not excreted promptly. A negative potassium balance did not occur until the third day of potassium chloride ingestion (Figure 4), and there was a positive potassium balance over the entire potassium chloride period (Table III).

In Experiment III (a patient with degenerative Bright's disease), the ingestion of 10 grams of potassium chloride per day resulted in relatively little potassium retention. By the second day of Period

II the potassium excretion almost equalled the intake and on the fourth day there was a negative potassium balance (Figure 6). Over the entire potassium chloride period there was a positive potassium balance of 11.8 m.eq. (Table V).

In Experiment IV (a patient with terminal hemorrhagic nephritis), the marked delay in excreting the added potassium reflected the patient's diminished kidney function. Not till the sixth day of Period II did the potassium excretion reach the intake (Figure 9), and over the eight



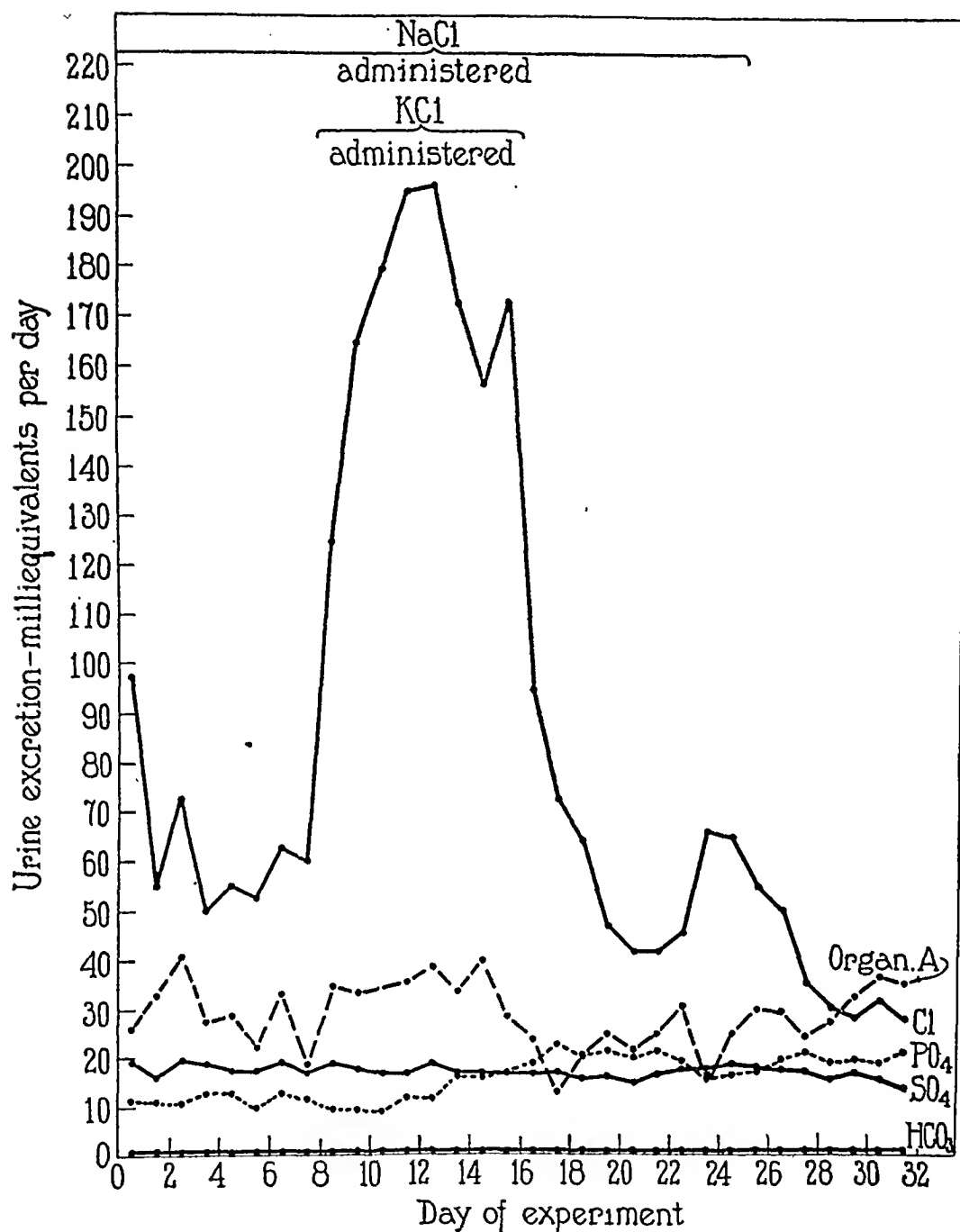


FIG. 8-b. URINARY EXCRETION OF ACID RADICLES, EXPERIMENT III

days of the period there was a retention of 28.4 m.eq. of potassium (Table VIII). This delay in excretion and the degree of potassium retention (Figure 7) is in marked contrast to the behavior observed in Experiment III.

2. The effect of potassium chloride ingestion on the sodium balance

(a) Normal subjects—Experiments I and II

Because of the failure to establish a constant level of sodium metabolism in Period I, Experiment I, the effect of KCl on the sodium balance

is not clear cut in Figure 1 and Table I. Nevertheless, it can be seen that the immediate effect of KCl ingestion was an increase in urinary sodium excretion to such an extent as to produce a marked negative sodium balance for the first two days of the KCl period (Figure 1). During the remaining four days of this period, however, sodium was retained. The retention of the last two days of the period was sufficient to compensate for the loss during the first two days, so that the average daily balance for Period II (Table I) was similar to that of Period I.

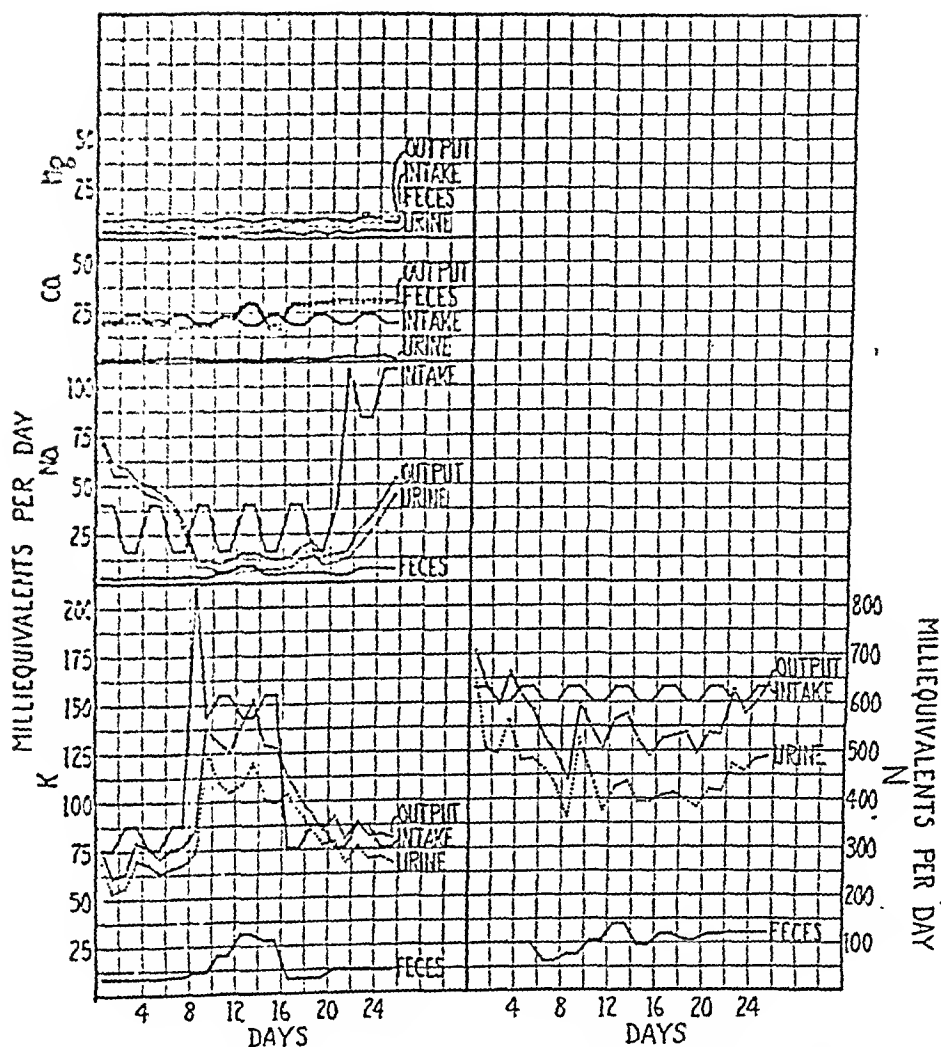


FIG. 9. DAILY BALANCES, EXPERIMENT IV, TERMINAL NEPHRITIC PATIENT

In contrast to Experiment I the subject in Experiment II attained approximate sodium equilibrium during Period I (Figure 4 and Table III). The ingestion of potassium chloride in Period II, Experiment II, was not accompanied by an immediate increase in urinary sodium excretion (Figure 4). However, on the third and fourth days of this period there was a definite increase in urinary sodium. The increased excretion was limited to these two days and, even during this time, the total sodium excretion did not exceed the sodium intake. The withdrawal of a 100 cc. blood sample was a factor in the negative sodium balance on the last day of the period. Column I, Table III, shows an average daily sodium balance of + 6.25

m.eq. for Period II, which is but slightly lower than that of Period I.

Thus in neither of the experiments on normal subjects did the ingestion of potassium chloride appreciably increase the sodium excretion over the entire period of potassium administration.

The detailed differences in the metabolic response in the two experiments furnish an example of the necessity of considering the previous dietary history and metabolic state of the body when interpreting clinical tests or studies on mineral and water metabolism (25).

In spite of differences in the preparation of the two normal subjects, the increase in the urinary sodium that followed the feeding of potassium

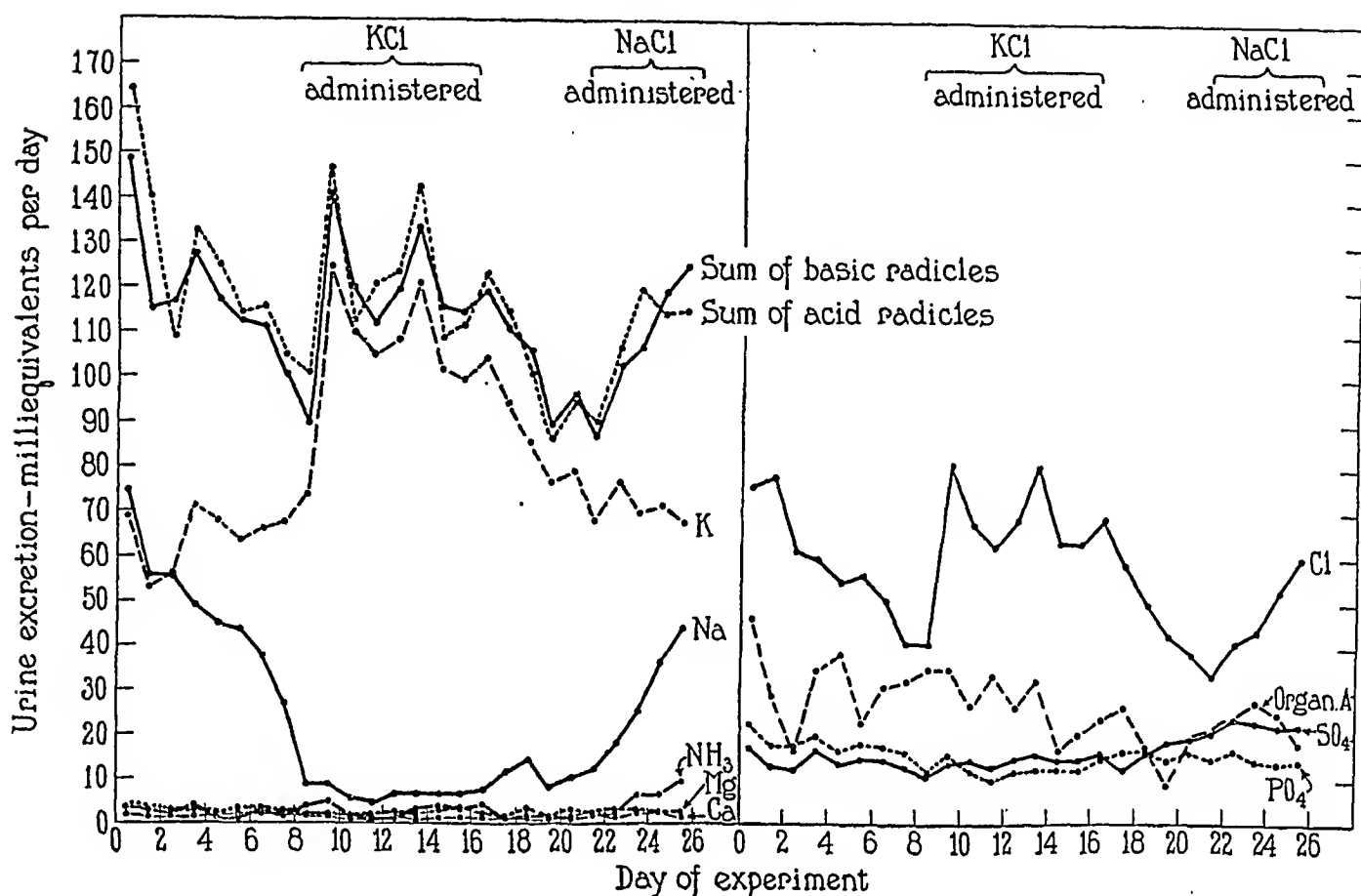


FIG. 10. URINARY EXCRETION OF BASIC AND ACID RADICLES, EXPERIMENT IV

chloride coincided in both with the establishment of the first negative potassium balance (Figures 1 and 4).

In Experiment II, the ingestion of the potassium chloride apparently affected the manner of sodium excretion more than the sodium balance, for the decrease in fecal sodium (Table IV) almost equalled the increase in urinary sodium. The total sodium excretion for Period II, Experiment II, was 90 per cent <sup>7</sup> of that of Period I, while the urinary and fecal sodiums were respectively 140 and 44 per cent of the respective sodium excretion of Period I. Possibly fecal sodium in Period I was high. But using Period III as a check, we find the fecal sodium of Period II diminished by 40 per cent.

(b) Nephritic subjects—Experiments III and IV

The data of Figure 6, Experiment III, show that this patient with degenerative Bright's dis-

ease had established an approximately stable level of sodium and potassium balance during Period I. Since the positive sodium balance over Period II was slightly in excess of that of Period I (Table V), the *ingestion of potassium chloride had no appreciable effect on the sodium retention and development of edema in the patient with nephrosis*. However, as in Experiments I and II, there was an increase in urinary sodium coincident to the establishment of the first negative potassium balance after the addition of potassium chloride (Figure 6).

The restriction of sodium chloride intake in Period IV resulted in a negative sodium balance and loss of weight, and thus was more effective in reducing edema than the administration of 10 grams of potassium chloride per day.

The data of Figure 9 and Table VIII (Experiment IV) show that this nephritic patient with terminal hemorrhagic nephritis had a marked loss of sodium during Period I when no KCl was given. This occurred over a period when there was no nausea, and probably reflects the limited ability that patients with markedly reduced func-

<sup>7</sup> The reduction of total sodium excretion simultaneous to a lesser positive sodium balance is made possible by a slightly lower sodium intake in Period II than in Period I due to the alternating diets.

TABLE I

Table of balances—Experiment I, normal Subject "B"

Period	Average balance per day					
	Sodium	Potassium	Calcium	Magnesium	Nitrogen	Nitrogen
	m.eq.	m.eq.	m.eq.	m.eq.	m.eq.	grams
I*	+26.8	+0.15	+6.58	-0.57	+68.4	+0.96
II	+28.2	-8.12	+6.68	-0.78	+77.8	+1.09
III	+36.2	-3.00	+4.70	-1.98	+71.5	+1.00
Entire experiment	+30.4	-3.66	+5.92	-1.11	+72.6	+1.02

\* Each period six days.

TABLE II

Relative excretion of ingested base and nitrogen in urine and feces. Experiment I, normal Subject "B," 68 kilos

		Period I	Period II	Period III	Average
Sodium....	Total intake per day, m.eq.	201	204	201	202
	Per cent of intake in urine	81	89	78	83
	Per cent of intake in feces	5.3	3.5	2.9	4.0
	Fecal excretion per day, m.eq.	11.0	7.5	5.9	8.2
Potassium...	Total intake per day, m.eq.	85	148	85	107
	Per cent of intake in urine	85	92	94	93
	Per cent of intake in feces	13.0	6.1	6.0	9.4
	Fecal excretion per day, m.eq.	11.0	9.1	7.5	9.3
Calcium....	Total intake per day, m.eq.	55	49	55	53
	Per cent of intake in urine	11	9	9	10
	Per cent of intake in feces	77	77	82	79
	Fecal excretion per day, m.eq.	42	38	45	42
Magnesium...	Total intake per day, m.eq.	33	31	33	32
	Per cent of intake in urine	25	29	27	27
	Per cent of intake in feces	76	73	79	76
	Fecal excretion per day, m.eq.	25	23	26	25
Nitrogen...	Intake per day, grams	19.7	19.7	19.7	19.7
	Per cent of intake in urine	8.8	7.0	7.7	7.8
	Fecal excretion per day, grams	1.74	1.38	1.52	1.55
	Grams fecal N X 70 Weight in kilos	1.79	1.42	1.57	1.60

tioning renal tissue have to limit sodium and chloride excretion when the salt intake is low, even though the serum sodium and chloride concentrations are below normal levels (26). Coincident with this negative sodium balance there was a marked loss of weight (Figure 7, Experiment IV) and a loss of edema.

The subsequent ingestion of the potassium chloride by this patient resulted in no increase in urinary sodium. Indeed, from the retention graph (Figure 7), it would seem as though the retention of potassium chloride had been accompanied by a retention of sodium. Though the daily dose of potassium chloride was small (5 grams), it was probably as large a dose as could safely be administered to this patient. We have,

therefore, in this experiment, as in Experiment III, evidence that potassium chloride administration is of less therapeutic value in treating edema than is sodium chloride restriction.

### 3. Correlation between base retention and weight changes

Analyses of serum base, sodium, and potassium in Experiments I and II, already published (27), showed variations from period to period hardly

TABLE III

Table of balances—Experiment II, normal Subject "M"

Period	Average balance per day					
	Sodium	Potassium	Calcium	Magnesium	Nitrogen	Nitrogen
	m.eq.	m.eq.	m.eq.	m.eq.	m.eq.	grams
I*	+7.54	-4.50	+1.83	-0.58	-57.80	-0.81
II*	+6.25	+5.83	+0.75	+1.18	+9.00	+0.13
III*	+14.47	-9.53	+1.35	+1.07	+87.00	+1.22
IV**	+18.57	-10.25	+0.22	+5.98	-36.20	-0.51
Total experiment	+11.08	-4.1	+1.13	+1.54	+3.82	+0.05

\* Six day period.

\*\* Four days instead of six.

TABLE IV

Relative excretion of ingested base and nitrogen in urine and feces. Experiment II, normal Subject "M," 70 kilos

		Period I	Period II	Period III	Period IV*	Average
Sodium....	Total intake per day, m.eq.	38	33	35	104	49
	Per cent of intake in urine	37	53	29	69	46
	Per cent of intake in feces	40	19	29	12	26
	Fecal excretion per day, m.eq.	15	6.4	11	12	11
Potassium...	Total intake per day, m.eq.	79	146	79	79	97
	Per cent of intake in urine	79	83	87	93	85
	Per cent of intake in feces	25	13	24	18	20
	Fecal excretion per day, m.eq.	21	18	19	14	18
Calcium....	Total intake per day, m.eq.	34	32	34	33	33
	Per cent of intake in urine	23	30	27	30	28
	Per cent of intake in feces	72	68	69	69	70
	Fecal excretion per day, m.eq.	24	22	23	23	23
Magnesium...	Total intake per day, m.eq.	37	35	37	37	37
	Per cent of intake in urine	49	48	51	45	49
	Per cent of intake in feces	54	49	45	33	45
	Fecal excretion per day, m.eq.	20	18	17	14	18
Nitrogen...	Intake per day, grams	15.7	15.4	15.7	15.5	15.6
	Per cent of intake in urine	11.0	10.0	11.0	9.3	10.0
	Fecal excretion per day, grams	1.82	1.54	1.55	1.44	1.53
	Grams fecal N X 70 Weight in kilos	1.82	1.54	1.55	1.44	1.53

\* Four days instead of six.

TABLE V  
Table of balances. Experiment III, nephrotic Patient "K"

Period	Average daily balance					
	Sodium	Potassium	Calcium	Magnesium	Nitrogen	Nitrogen
	m.eq.	m.eq.	m.eq.	m.eq.	m.eq.	grams
I.....	+44.20	- 2.42	+0.19	-3.14	+73.4	+1.03
II.....	+47.10	+11.80	-4.34	-8.78	+57.5	+0.80
III *....	+60.80	- 5.61	+4.14	+1.23	+93.8	+1.31
IV **....	- 7.14	- 6.53	-2.53	+4.30	+37.7	+0.53
Total....	+38.40	- 0.66	-0.43	-1.69	+67.4	+0.94

\* 9 days instead of 8.  
\*\* 7 days instead of 8.

greater than the experimental errors of the methods. It, therefore, appears probable that changes in concentration of extracellular body fluids did not account for any important part of the sodium and potassium retentions and losses in Experiments I and II.

During the first eleven days of Experiment I there was a close correlation between the sum of sodium and potassium retentions and weight changes corresponding to the ratio of 150 milliequivalents of base per kilo weight change (Figure 2). The data indicate an approximate constancy of base concentration in the body fluids and a major part of the weight changes to be due to changes in extracellular body fluid volume.

Over the last seven days of Experiment I (Figure 2) the correlation between base retention and weight change ceased, base retention occurring without an equivalent weight increase. This behavior exemplifies the result of an acute upper respiratory infection. The subject developed a severe cold on the thirteenth day of the experiment. On the fourteenth day he had a sore throat and on the fifteenth day went to bed with a fever.

TABLE VI  
Relative excretion of ingested base and nitrogen in urine and feces. Experiment III, nephrotic Patient "K," 59 kilos

		Period I	Period II	Period III	Period IV	Average
Sodium....	Total intake per day, m.eq.	102	102	102	33	87
	Per cent of intake in urine	45	42	27	57	42
	Per cent of intake in feces	11	12	13	64	23
	Fecal excretion per day, m.eq.	12	12	13	21	14
Potassium..	Total intake per day, m.eq.	70	204	70	69	103
	Per cent of intake in urine	94	87	96	91	92
	Per cent of intake in feces	9.2	7.4	12.0	18	11
	Fecal excretion per day, m.eq.	6.4	15.0	8.1	12	10
Calcium....	Total intake per day, m.eq.	23	23	23	23	23
	Per cent of intake in urine	21	7.4	3.8	9.2	10
	Per cent of intake in feces	78	112	78	96	91
	Fecal excretion per day, m.eq.	18	26	18	22	21
Magnesium..	Total intake per day, m.eq.	20	20	20	19	20
	Per cent of intake in urine	22	14	14	22	18
	Per cent of intake in feces	94	130	80	56	91
	Fecal excretion per day, m.eq.	18	26	16	11	18
Nitrogen...	Intake per day, grams	9.44	9.44	9.42	9.50	9.44
	Per cent of intake in feces	18.00	22.00	19.00	20.00	20.00
	Fecal excretion per day, grams	1.69	2.09	1.77	1.89	1.85
	Grams fecal N $\times$ 70					
	Weight in kilos	2.01	2.48	2.10	2.24	2.20

This event gave an unexpected opportunity for observing the inorganic metabolism during the onset of such an infection. The retention of base over this period was not accompanied by a parallel increase in weight, apparently because an increase in sodium retention occurred without a corresponding increase in extracellular fluid. The retention of sodium was similar to that of chloride reported by Sunderman (28) and of fixed base by Austin and Sunderman (29) in studies on pneumonia patients receiving a liberal sodium chloride intake.

During Period I of Experiment II the retention data (Figure 2) show a correlation between the sum of the potassium and sodium retentions and weight change. During the KCl period, i.e. Pe-

TABLE VII  
Characterization of edema of nephrotic Patient, "K," Experiment III

Period *	Arms	Face	Legs	Scrotum	Back	Thorax	Abdomen
Fore.....	0	+	++	0	+	±	±
I.....	+	++	++++	+	+	++	++
II.....	++	+++	+++++	++	++++	+++	+++++
III.....	+++	++++	+++++	+++	+++++	+++	+++++
IV.....	++	++++	+++++	++	+++++	++	+++++

\* Characterization at end of each period given.

TABLE VIII

Table of balances. Experiment II\*, nephritic Subject "L."

Period	Average daily balance					
	Sodium	Potassium	Calcium	Magnesium	Nitrogen	Nitrogen
	m.eq.	m.eq.	m.eq.	m.eq.	m.eq.	grams
I.....	-23.3	+ 8.60	+1.80	+2.78	+14.1	+0.20
II.....	+15.8	+28.40	-0.95	+2.99	+85.5	+1.20
III.....	+15.1	-17.00	-9.00	+0.12	+95.4	+1.34
IV.....	+64.6	- 2.76	-5.56	-1.24	+24.2	+0.34
Total....	+13.0	+ 7.58	-2.54	+1.94	+63.6	+0.75

\* 5 days instead of 8.

TABLE IX

Relative excretion of ingested base and nitrogen in urine and feces. Experiment IV, nephritic Patient "L," 57 kilos

		Period I	Period II	Period III	Period IV	Average
Sodium....	Total intake per day, m.eq.	25	25	31	52	42
	Percent of intake in urine	170	24	34	25	57
	Percent of intake in feces	12.0	19.0	16.0	6.5	11.0
	Fecal excretion per day, m.eq.	3.3	5.4	4.5	6.4	4.8
Potassium....	Total intake per day, m.eq.	82	137	81	81	104.5
	Percent of intake in urine	79	67	110	88	79
	Percent of intake in feces	11	15	11	15	13
	Fecal excretion per day, m.eq.	8.7	23	9.2	12	14
Calcium....	Total intake per day, m.eq.	21	21	21	21	21
	Percent of intake in urine	7.4	5.4	6.6	9.8	7.0
	Percent of intake in feces	84	93	135	140	109
	Fecal excretion per day, m.eq.	18	21	29	29	23
Magnesium....	Total intake per day, m.eq.	8.8	8.8	8.7	8.7	8.8
	Percent of intake in urine	31.0	26.0	28.0	33.0	29.0
	Percent of intake in feces	37.0	40.0	70.0	81.0	53.0
	Fecal excretion per day, m.eq.	3.3	3.5	6.1	7.0	4.6
Nitrogen....	Intake per day, grams	8.6	8.6	8.7	8.7	8.6
	Percent of intake in feces	15.0	17.0	18.0	19.0	17.0
	Fecal excretion per day, grams	1.3	1.5	1.6	1.7	1.5
	Grams fecal N $\times$ 70 Weight in kilos	1.60	1.64	1.56	2.00	1.54

riod II, the increase in weight was greater than could be accounted for by the retention of base with a concentration of 150 milliequivalents per liter of body fluid. In the absence of a decrease in plasma sodium and potassium (27), this lack of correlation between base and acid retentions may have been due to a dilution of intracellular base. During Period III there was a decrease in weight without a corresponding decrease in base. The excess loss in weight over base in Period III approximately equalled the excess increase in weight over base retention in Period II. In Period IV

the retention of sodium and increase in weight showed a correlation indicative of extracellular fluid retention.

The retention data of Experiment III (Figure 7) show a close correlation between base retention and weight changes on the basis of 150 milliequivalents of base per liter of body fluid. Since the sodium and base retentions were very nearly the same, except over Period II, and since the correlation between sodium retentions and weight changes was as good as that between base retention and weight change, the data indicate that the weight changes in this experiment are due to extracellular fluid changes, unless there has been a change in the permeability of cell membranes to sodium (30, 31).

The retention data of Experiment IV (Figure 7) show some correlation between sodium retention and weight change, but no such clear correlation as shown in Experiment III. There is no clear relation between potassium retention and weight change. Nor is there any discernible relation between the retention of sodium plus potassium and weight change that explains the discrepancies between base retention and water balance. Since we did not have plasma base analyses on this patient we cannot consider the effects of changing plasma base concentrations on the water balance. The balance data, however, would suggest plasma concentration changes, and support the known fact that such patients are unable to maintain normal base concentrations.

#### 4. Urinary excretion of acids and bases

The approximate constancy of the urinary ammonia and bicarbonate in Experiments I, II, and III indicate that the KCl caused little if any disturbance of the acid-base equilibrium such as has been reported by others (10). In Experiment IV the relatively constant urinary bicarbonate,<sup>8</sup> pH, and ammonia is not necessarily an index of little disturbance in acid-base balance inasmuch as it may reflect the inability of the kidneys to alter the concentrations of these urinary constituents to any great extent.

The sum of the total cations and sum of the

<sup>8</sup> The pH of these urines varied from 5.3 to 5.4 and at this acidity the bicarbonate was so low that it has been omitted from Figure 10.



# STUDIES ON THE ANEMIA OF PELLAGRA

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Extensive studies limited to the anemia of endemic pellagra were made in 1923 by Huck (1) and in 1933 by Turner (2). These investigators observed that anemia did not occur frequently in this type of pellagra, but, if present, was usually of a mild degree with a low color index and microcytosis; macrocytosis was not noted.

The pellagra occurring in the northern part of the United States has been termed "pseudo-pellagra," "alcoholic pellagra" or "pseudo-alcoholic pellagra" (3, 4, 5, 6) in contrast to the endemic type found in the southern part of the United States. Spies and DeWolf (7), however, showed that alcoholic and endemic pellagra are similar. The essential difference between the two types is that in "alcoholic pellagra" the patient loses the desire for food and lives almost exclusively from the calories in the ingested alcohol. In endemic pellagra, on the other hand, the patient receives a diet which is more complete but still deficient in some necessary nutritional constituents. They recognized, moreover, that "alcoholic pellagra" is in general more acute and more often fatal; this may be due to the fact that it follows almost complete abstinence from food.

Since so few determinations of blood values had been made on individuals who developed pellagra secondary to alcoholism, it seemed worth while to make the following hematological studies.

## METHODS

Red and white blood cell counts, hemoglobin determinations, and estimations of mean corpuscular volume were performed twice a week or oftener, if indicated, on each of 30 consecutive cases of severe pellagra admitted to the Lakeside Hospital for treatment. While from 10 to 46 hematological studies were made by the authors on each of the 30 pellagrins, the blood values in the table were obtained by averaging the first two determinations. Pipettes certified for accuracy by the U. S. Bureau of Standards were used; the

cells were counted by the usual technique. The hemoglobin determinations were made by the Sahli acid hematin method, using standards which were checked by the Van Slyke and Neill method (8) for determination of oxygen capacity. The mean corpuscular volume was calculated by the hematocrit method of Van Allen (9). These procedures were further standardized by repeated observations on 10 adults in good health. The pipettes, calibrated tubes, hemoglobinometers, and hemocytometers were the same as those used for the pellagrins. The only difference in the two studies was that the observations on the individuals with pellagra were continued for a longer period of time. After the completion of all blood determinations on the 30 patients and the 10 individuals serving as controls, the color index, volume index, mean corpuscular hemoglobin in micromicrograms, mean corpuscular volume in cubic microns, and the saturation index were calculated. In all instances 15.5 grams hemoglobin were regarded as 100 per cent, and all persons with a hemoglobin value below 80 per cent and a red blood cell count of 4 million or less were arbitrarily considered as having anemia.

Repeated gastric analyses were done on only 27 of the 30 pellagrins; the remaining 3 were considered too ill. Breakfast was withheld the morning of the test, a small Rehfuß tube was passed, and the fasting contents of the stomach were withdrawn. Immediately after this procedure, 0.0005 gram ergamine acid phosphate was injected subcutaneously. Twenty minutes later and again 40 minutes later the contents of the stomach were once more withdrawn. Free and total acidity were determined in the usual way, N/10 sodium hydroxide being used for titration and Topfer's reagent and phenolphthalein as indicators. A quantitative estimate of pepsinogen was made by the Mett tube method, and the presence of rennin was determined by incubation of a mixture of 5 drops of gastric juice and 5 cc. of milk for 30 minutes at 37° C.





injection of ergamine acid phosphate but even with the increase, the majority of cases did not have so much as 20 cc. gastric juice which could be withdrawn from the stomach. In this small series of cases the degree of anemia did not seem to be significantly related to the degree of hypochlorhydria or achlorhydria.

#### DISCUSSION

It has been shown in the course of this investigation that 63.3 per cent of the severely ill pellagrins had an anemia, which can be classified into the following 2 types: (1) about 75 per cent of the patients with anemia had definite increase in the volume of the red blood cells and a color index of 1 or above; (2) the other 25 per cent had an anemia characterized by a decrease in the red blood cell volume and color index. Since all of these patients with pellagra are known to have eaten only small amounts of food for a long period of time, it seems likely that they suffered from the lack of many necessary food substances, including iron and the antianemic substance found in liver.

It is theoretically conceivable that the anemia associated with pellagra may be caused by one of the following factors or by some combination of these factors:

1. Dysfunction of the stomach.
2. Failure of adequate ingestion of iron or other nutritional substances important in erythropoiesis.
3. Possible hepatic changes interfering with storage of the antianemic factor.

Any attempt, however, to correlate the presence of anemia in pellagrins with the above factors gives rise to various difficulties. In the first place, it is important to realize that all pellagrins do not develop anemia. This is true regardless of whether they give a history of small food intake or heavy drinking over a period of many years, or whether they have achylia gastrica. Since this is the case, it is possible that some quantitative relationship may exist between some of the aforementioned factors and the development of anemia. In the second place, the administration of any one specific therapeutic agent such as yeast, liver extract, or iron did not invariably bring about a uniform hematological response in those patients who did develop anemia. Further studies must be made before this latter mechanism can be under-

stood, but at the present it has been shown that the pellagrins who developed anemia eventually attained normal blood values when given antipellagric therapy over a long period of time. The relationship of achylia gastrica to the development of anemia in pellagrins may be of some quantitative importance, but the studies of Spies and Payne (12) showed that the gastric juice from 2 pellagrins activated beef in such a manner as to cause a remission of pernicious anemia, thus demonstrating that the unknown constituent of the gastric juice, which is lacking in the pernicious anemia patient, was present in the stomach contents of the pellagrins. The possible relationship between hepatic changes and hemopoiesis in these cases is at present only a theoretical concept. It may be of some importance, however, that post-mortem examination of the livers from the patients who have died in this clinic frequently showed fatty infiltration (13).

#### CONCLUSIONS

1. It has been shown in this study that the peripheral blood findings of an anemia, usually characterized by increased red blood cell volume, occur in 63.3 per cent of 30 severely diseased "alcoholic" pellagrins.

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TABLE I  
Blood values of thirty cases of alcoholic pellagra

Date	Number	Name	Hospital number	Age	Sex	Race	Free gastric acidity after histamine	R.B.C.	Hemoglobin	Hemoglobin	Hematocrit	Color index	Mean corpuscular hemoglobin	Volume index	Mean corpuscular volume	Saturation index
				years			cc. N/10 per 100	millions	per cent	grams per cent	per cent		micro-micrograms		cubic microns	
June 8, 1933	1.	C. F.	133089	49	M.	W.	0	3.79	82	13.0	45	1.09	35.1	1.33	121	.83
June 11, 1933	2.	W. M.	152770	70	M.	W.	0	4.47	74	11.4		.87	26.1			
June 27, 1933	3.	J. C.	152763	42	M.	W.	0	3.85	85	13.7	44	1.10	36.2	1.25	116	.80
June 30, 1933	4.	H. D.	122436	43	M.	W.		2.93	60	9.69	29	1.04	33.1	1.09	100	.95
July 3, 1933	5.	R. H.	152893	42	M.	W.	39	3.73	78	12.9	38	1.06	35.1	1.12	103	.94
July 3, 1933	6.	A. S.	135696	26	F.	C.	5	3.46	78	12.1	43	1.13	34.8	1.35	123	.84
July 3, 1933	7.	R. R.	153011	36	F.	C.	0	3.35	68	10.3	34	1.01	31.9	1.10	103	.92
July 6, 1933	8.	C. A.	152207	43	M.	W.	0	3.71	71	10.6	37	.96	28.5	1.08	100	.88
July 6, 1933	9.	W. C.	153208	31	F.	C.	0	3.77	68	11.0		.92	29.9			
July 7, 1933	10.	A. M.	153224	44	M.	W.	0	3.71	78	12.1	44	1.05	32.7	1.28	119	.82
July 11, 1933	11.	M. C.	153286	44	M.	C.	0	3.56	80	12.8	42	1.13	36.6	1.31	120	.80
July 17, 1933	12.	A. C.	144625	36	F.	C.	0	3.46	83	12.9	43	1.20	37.1	1.35	123	.74
July 27, 1933	13.	R. S.	153568	32	M.	C.	0	4.21	82	13.0		1.00	30.9			
August 1, 1933	14.	C. L.	153454	51	M.	C.	20	3.19	72	10.9	35	1.14	34.0	1.20	113	.94
August 1, 1933	15.	J. T.	153620	48	M.	W.	18	3.65	82	12.4	42	1.12	33.5	1.22	114	.90
August 16, 1933	16.	W. R.	137902	48	M.	C.	40	4.18	73	11.0	42	.88	25.7	1.08	100	.80
August 18, 1933	17.	M. W.	153944	36	F.	W.	58	3.68	78	12.4	42	1.07	33.5	1.25	114	.85
August 18, 1933	18.	C. W.	143320	26	F.	C.	12	3.03	75	11.6	41	1.25	38.7	1.50	136	.80
August 24, 1933	19.	B. M.	152573	30	F.	W.	5	4.71	90	14.2	44	.97	30.0	1.02	94	.94
September 11, 1933	20.	O. Q.	154338	38	F.	C.		4.48	80	12.4		.91	28.2			
September 23, 1933	21.	G. P.	154544	44	M.	W.	0	3.13	71	11.2	42	1.13	36.1	1.46	136	.80
November 18, 1933	22.	S. L.	155469	15	M.	W.	23	4.75	84	13.0		.90	27.7			
November 20, 1933	23.	G. W.	155481	29	M.	C.	0	3.94	73	10.2		.93	26.2			
November 22, 1933	24.	A. Y.	153313	31	F.	C.	0	3.15	70	10.0	38	1.09	32.2	1.28	123	.85
December 1, 1933	25.	E. H.	155669	38	F.	C.	21	3.84	60	9.9		.79	26.1			
December 8, 1933	26.	W. T.	149182	28	M.	C.	0	4.71	85	12.1	44	.90	25.7	1.02	94	.90
January 16, 1934	27.	G. E.	156351	47	M.	C.		5.47	89	14.3		.85	26.5			
January 23, 1934	28.	E. S.	153688	29	F.	C.	0	4.28	79	13.2		.93	31.3			
February 10, 1934	29.	A. L.	149424	41	M.	C.	0	4.82	94	15.6	46	.98	32.5	1.04	96	.94
February 10, 1934	30.	F. C.	156057	51	M.	C.	0	4.99	97	13.7	45	.99	27.9	1.00	95	.99

## OBSERVATIONS

Nineteen of the 30 patients, 63.3 per cent, had an anemia with an average red cell count of 3.5 million and an average hemoglobin value of 74 per cent (see Table I). Of these 19, 15 had a color index averaging 1.11 (see Figure 1) and a volume index above 1. Identical determinations made on the individuals used as controls gave an average color index of 0.98 and an average volume index of 1.04 (consistent with the normal values of Haden (10) and Osgood (11)).

Seventeen of the 30 pellagrins had achylia gastrica as evidenced by the absence of free HCl, pepsinogen, and rennin following the administration of ergamine acid phosphate. Five of the remaining 10 pellagrins on whom gastric analyses were performed during the acute stages of the disease had definite hypoacidity and decreased values of pepsinogen and rennin. The gastric

juice in nearly all instances was markedly reduced in volume, nearly always being less than 20 cc. Oftentimes it appeared to be entirely mucus, but occasionally it was free-flowing and limpid. The volume of gastric secretion increased following the

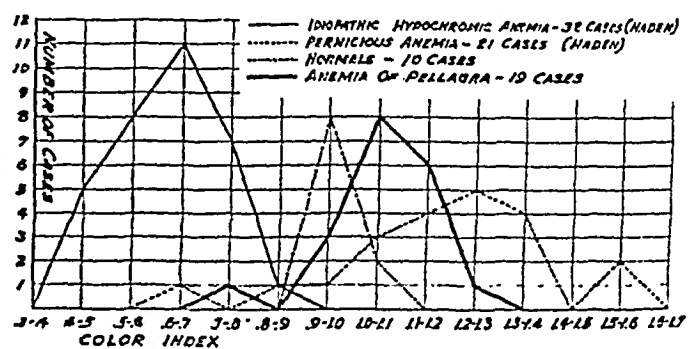


FIG. 1. SHOWING HOW A CURVE REPRESENTING THE COLOR INDEX VALUES OF THE ANEMIA OF ALCOHOLIC PELLAGRA LIES NEAR THE NORMAL AND BETWEEN SIMILAR CURVES REPRESENTING THOSE OF IDIOPATHIC HYPOCHROMIC ANEMIA AND PERNICIOUS ANEMIA.

injection of ergamine acid phosphate but even with the increase, the majority of cases did not have so much as 20 cc. gastric juice which could be withdrawn from the stomach. In this small series of cases the degree of anemia did not seem to be significantly related to the degree of hypochlorhydria or achlorhydria.

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1. Dysfunction of the stomach.
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Any attempt, however, to correlate the presence of anemia in pellagrins with the above factors gives rise to various difficulties. In the first place, it is important to realize that all pellagrins do not develop anemia. This is true regardless of whether they give a history of small food intake or heavy drinking over a period of many years, or whether they have achylia gastrica. Since this is the case, it is possible that some quantitative relationship may exist between some of the aforementioned factors and the development of anemia. In the second place, the administration of any one specific therapeutic agent such as yeast, liver extract, or iron did not invariably bring about a uniform hematological response in those patients who did develop anemia. Further studies must be made before this latter mechanism can be under-

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				years			cc. N/10 per 100	millions	per cent	grams per cent	per cent		micro-micro-grams		cubic microns	
June 8, 1933	1.	C. F.	133089	49	M.	W.	0	3.79	82	13.0	45	1.09	35.1	1.33	121	.83
June 11, 1933	2.	W. M.	152770	70	M.	W.	0	4.47	74	11.4		.87	26.1			
June 27, 1933	3.	J. C.	152763	42	M.	W.	0	3.85	85	13.7	44	1.10	36.2	1.25	116	.80
June 30, 1933	4.	H. D.	122436	43	M.	W.		2.93	60	9.69	29	1.04	33.1	1.09	100	.95
July 3, 1933	5.	R. H.	152893	42	M.	W.	39	3.73	78	12.9	38	1.06	35.1	1.12	103	.94
July 3, 1933	6.	A. S.	135696	26	F.	C.	5	3.46	78	12.1	43	1.13	34.8	1.35	123	.84
July 3, 1933	7.	R. R.	153011	36	F.	C.	0	3.35	68	10.3	34	1.01	31.9	1.10	103	.92
July 6, 1933	8.	C. A.	152207	43	M.	W.	0	3.71	71	10.6	37	.96	28.5	1.08	100	.88
July 6, 1933	9.	W. C.	153208	31	F.	C.	0	3.77	68	11.0		.92	29.9			
July 7, 1933	10.	A. M.	153224	44	M.	W.	0	3.71	78	12.1	44	1.05	32.7	1.28	119	.82
July 11, 1933	11.	M. C.	153286	44	M.	C.	0	3.56	80	12.8	42	1.13	36.6	1.31	120	.80
July 17, 1933	12.	A. C.	144625	36	F.	C.	0	3.46	83	12.9	43	1.20	37.1	1.35	123	.74
July 27, 1933	13.	R. S.	153568	32	M.	C.	0	4.21	82	13.0		1.00	30.9			
August 1, 1933	14.	C. L.	153454	51	M.	C.	20	3.19	72	10.9	35	1.14	34.0	1.20	113	.94
August 1, 1933	15.	J. T.	153620	48	M.	W.	18	3.65	82	12.4	42	1.12	33.5	1.22	114	.90
August 16, 1933	16.	W. R.	137902	48	M.	C.	40	4.18	73	11.0	42	.88	25.7	1.08	100	.80
August 18, 1933	17.	M. W.	153944	36	F.	W.	58	3.68	78	12.4	42	1.07	33.5	1.25	114	.85
August 18, 1933	18.	C. W.	143320	26	F.	C.	12	3.03	75	11.6	41	1.25	38.7	1.50	136	.80
August 24, 1933	19.	B. M.	152573	30	F.	W.	5	4.71	90	14.2	44	.97	30.0	1.02	94	.94
September 11, 1933	20.	O. O.	154338	38	F.	C.		4.48	80	12.4		.91	28.2			
September 23, 1933	21.	G. P.	154544	44	M.	W.	0	3.13	71	11.2	42	1.13	36.1	1.46	136	.80
November 18, 1933	22.	S. L.	155469	15	M.	W.	23	4.75	84	13.0		.90	27.7			
November 20, 1933	23.	G. W.	155481	29	M.	C.	0	3.94	73	10.2		.93	26.2			
November 22, 1933	24.	A. Y.	153313	31	F.	C.	0	3.15	70	10.0	38	1.09	32.2	1.28	123	.85
December 1, 1933	25.	E. H.	155669	38	F.	C.	21	3.84	60	9.9		.79	26.1			
December 8, 1933	26.	W. T.	149182	28	M.	C.	0	4.71	85	12.1	44	.90	25.7	1.02	94	.90
January 16, 1934	27.	G. E.	156351	47	M.	C.		5.47	89	14.3		.85	26.5			
January 23, 1934	28.	E. S.	153688	29	F.	C.	0	4.28	79	13.2		.93	31.3			
February 10, 1934	29.	A. L.	149424	41	M.	C.	0	4.82	94	15.6	46	.98	32.5	1.04	96	.94
February 10, 1934	30.	F. C.	156057	51	M.	C.	0	4.99	97	13.7	45	.99	27.9	1.00	95	.99

OBSERVATIONS

Nineteen of the 30 patients, 63.3 per cent, had an anemia with an average red cell count of 3.5 million and an average hemoglobin value of 74 per cent (see Table I). Of these 19, 15 had a color index averaging 1.11 (see Figure 1) and a volume index above 1. Identical determinations made on the individuals used as controls gave an average color index of 0.98 and an average volume index of 1.04 (consistent with the normal values of Haden (10) and Osgood (11)).

Seventeen of the 30 pellagrins had achylia gastrica as evidenced by the absence of free HCl, pepsinogen, and rennin following the administration of ergamine acid phosphate. Five of the remaining 10 pellagrins on whom gastric analyses were performed during the acute stages of the disease had definite hypoacidity and decreased values of pepsinogen and rennin. The gastric

juice in nearly all instances was markedly reduced in volume, nearly always being less than 20 cc. Oftentimes it appeared to be entirely mucus, but occasionally it was free-flowing and limpid. The volume of gastric secretion increased following the

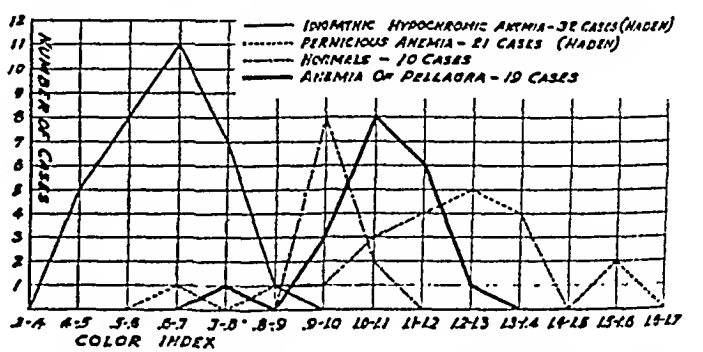


FIG. 1. SHOWING HOW A CURVE REPRESENTING THE COLOR INDEX VALUES OF THE ANEMIA OF ALCOHOLIC PELLAGRA LIES NEAR THE NORMAL AND BETWEEN SIMILAR CURVES REPRESENTING THOSE OF IDIOPATHIC HYPOCHROMIC ANEMIA AND PERNICIOUS ANEMIA.

# THE ACTION OF DINITROPHENOL AND INSULIN IN ACCELERATING THE METABOLISM OF ETHYL ALCOHOL<sup>1</sup>

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The value, on occasion, of accelerating the removal of alcohol from the body, especially in cases of acute alcoholic intoxication, is so evident as to require no elaboration. However, the amount which can be eliminated by the ordinary excretory channels is so small as to be practically insignificant, and the rate of metabolism of alcohol in the body is peculiarly resistant to modification, proceeding at a constant rate (1, 2, 3) for the individual. In spite of the popular sobering effect of a brisk walk in the cold air, it has been shown experimentally that neither external cold (4) nor muscular exercise (5, 6, 7) exerts the slightest influence on this constant rate of metabolism.

Nevertheless, two substances, dinitrophenol and insulin, have been demonstrated to accelerate the speed of combustion of alcohol in experimental animals. It was shown by Supniewski (8) that the simultaneous administration of a unit of insulin per kilogram with the dose of alcohol in rabbits caused twice as rapid a fall of blood alcohol as in controls which did not receive insulin. Insulin is also known to increase the acetaldehyde concentration of the blood (8), and acetaldehyde may be one of the intermediary products in the metabolism of alcohol. Widmark (9) has shown that the administration of 10 mgm. per kilogram of dinitrophenol to dogs which have received a dose of alcohol is effective in more than doubling the rate of disappearance of alcohol from the blood. He feels that at least a part of this increase may be due to hyperventilation and hyperthermia which accompany the dose employed. This work has been confirmed by Harger and Hulpieu (10).

It has been the purpose of the present work to ascertain the value of these agents in the practi-

cal therapeutics of alcohol intoxication in man. To this end, we have subjected them to experimental trial both in vitro and in vivo, always bearing in mind that the dosage used must be within the limits prescribed by safety in administration to man.

## EXPERIMENTAL WORK

The action of dinitrophenol in accelerating the metabolism of alcohol by rat liver in vitro has already been reported by us (11). Suffice it to say that concentrations of from 1:5,000,000 to 1:20,000,000 were shown to increase the amount of .1 per cent alcohol metabolized by a fixed amount of rat liver from 5 to 10 per cent. Higher concentrations showed no effect or slight inhibition. In the absence of animal tissue, dinitrophenol was not effective in causing a disappearance of alcohol from the solution.

In vivo, two approaches were made to the problem. Firstly, a test dose of .5 cc. ethyl alcohol was administered to a subject, and the rate of fall of the alcohol content of the blood followed at intervals. Subsequently, the subject was given 100 mgm. dinitrophenol three times daily for a period of two weeks, during which time there was an increase of 30 per cent in her basal metabolic rate. In spite of this, on the administration of a similar test dose the rate of fall of the blood alcohol was found to be identical with the first trial.

Secondly, another subject was given 1.5 cc. of alcohol per kilogram as a 20 per cent solution in normal saline in one hour, the intravenous route being again employed. An hour was allowed to elapse for equilibration between the blood and tissues, and then the rate of decline of the blood alcohol followed at hourly intervals for two hours, at the end of which time 500 mgm. of dinitrophenol were administered by mouth, and the blood alcohol again determined at hourly in-

<sup>1</sup> Supported in part by a grant from the Rockefeller Fluid Research Fund of Stanford University School of Medicine.

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of insulin per kilogram was administered hypodermically, the simultaneous administration of 200 grams of glucose in a small amount of water being effected by mouth. Hourly blood samples were analyzed for alcohol subsequent to the injection. At no time did the subjects evidence any of the signs of hypoglycemic shock. Figure 1 shows the acceleration of the decline of the alcohol curve following the administration of insulin in one of the subjects, amounting to a 50 per cent increase in the rate. The increase in rate of metabolism in each case is seen in Table I.

In order to determine if the insulin effect could be produced by the insulin free pancreatic extract, the experiment was repeated, 10 cc. of the extract being given intramuscularly in place of the insulin. The results are tabulated in Table I, and in the case of one of the subjects, shown graphically in Figure 1, show about the same activity as insulin.

#### SUMMARY

It has been shown that both dinitrophenol and the two pancreatic extracts are capable of increasing the rate of metabolism of alcohol by rat liver *in vitro*. Insulin and insulin-free pancreatic extract, unlike dinitrophenol, are effective in causing the oxidation of alcohol in the absence of animal tissue.

*In vivo*, dinitrophenol was not effective in appreciably increasing the rate of oxidation of alcohol in the maximum dose deemed safe for routine administration in man. Insulin, however, in a dose of 1 unit per kilogram, is effective in producing a fifty per cent increase in the rate of disappearance of alcohol from the blood stream. When administered with the appropriate amount of glucose, this dosage may be used safely. That the action may not be due to insulin itself, but to some other component of the commercial product, is shown by the similar effect demonstrated with the insulin-free pancreatic extract. Indeed, it would be very unlikely that a substance such as insulin, with its highly specific action, should also be a factor in alcohol metabolism. Much more probable is the presence in pancreatic extract, and perhaps in the extracts of other organs, of a principle capable of effecting the combustion of alcohol. The isolation of this principle from the other components of tissue extracts, and its con-

centration, may give us a preparation of value in acute alcoholic intoxication. Further work in this direction is in progress.

#### CONCLUSIONS

1. Dinitrophenol accelerates the metabolism of alcohol by animal tissues, *in vitro*.
2. Insulin and insulin-free pancreatic extract effect the oxidation of alcohol *in vitro* in the absence of tissue.
3. Dinitrophenol is not an effective accelerator of alcohol metabolism in safe doses in man.
4. Insulin and insulin-free pancreatic extract are capable of increasing the rate of alcohol metabolism approximately fifty per cent in therapeutic doses in man.
5. Isolation of the principle responsible for this action may yield a useful accelerator of alcohol metabolism free from undesirable side-actions.

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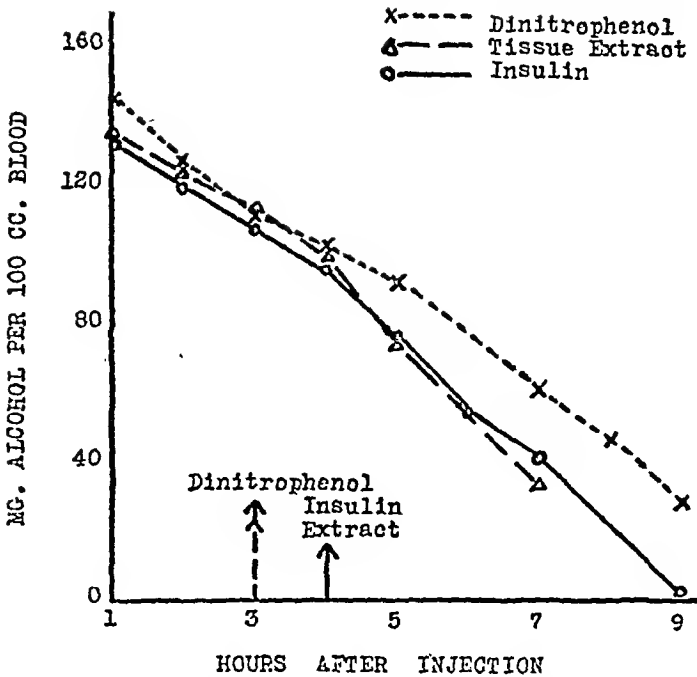


FIG. 1. THE INFLUENCE OF DINITROPHENOL, INSULIN, AND INSULIN-FREE PANCREATIC EXTRACT ON THE CURVE OF BLOOD ALCOHOL CONCENTRATION FOLLOWING ADMINISTRATION OF A TEST DOSE OF ALCOHOL INTRAVENOUSLY.

tervals. The results are shown in Figure 1, and tabulated in Table I. No rise in body temperature, or noticeable increase in respiration, was observed with this dose, which is, however, as high as could be routinely administered to man with safety.

TABLE I

Increase in rate of metabolism of alcohol in man

Accelerator	Average decline before administration	Average decline after administration	Increase
	mgm. per 100 cc. per hour	mgm. per 100 cc. per hour	per cent
Dinitrophenol, 500 milligrams.....	13.7	14.4	5
Insulin, 1 unit per kilogram.....	11.7	18.0	54
Insulin, 1 unit per kilogram.....	14.3	20.0	40
Tissue extract, 10 cc.....	11.3	18.6	65
Tissue extract, 10 cc.....	14.3	16.7	17

With insulin, the same technic in vitro was employed as with dinitrophenol. Three hundred milligrams of rat liver, sliced to a thickness of .5 mm., were placed in a 25 cc. Ehrlenmeyer flask, 3 cc. of approximately .1 per cent alcohol in phos-

phate buffer at pH 7.4 and sufficient insulin to bring the solution to the desired concentration added, the air replaced by oxygen, and the flask agitated 120 times per minute in an incubator at 37 degrees C. for two hours. At the end of this time the action was stopped by placing the flask in ice water, and the analyses for alcohol carried out by the method of Cannan and Sulzer (12). The results may be seen in Table II. Controls were run in each case without insulin. All values are the average of duplicate determinations.

TABLE II

Increase in alcohol combustion in vitro

Preparation (3 cc. 0.1 per cent alcohol in buffer pH 7.4 in each)	Increase in alcohol combustion over control mgm. per 100 cc. per hour
Liver and insulin, 1 unit per liter.....	5.0
Liver and insulin, 1 unit per liter.....	2.6
Insulin, 1 unit per liter.....	5.5
Insulin, 1 unit per liter.....	2.7
Insulin, 1 unit per liter.....	2.9
Insulin, 1 unit per liter.....	3.2
Insulin, 1 unit per liter.....	2.2
Tissue extract, 0.5 cc. per liter.....	2.7
Tissue extract, 0.5 cc. per liter.....	1.9

Again a control was run on the same mixture without the presence of the rat liver. Here, in contrast to the inactivity of dinitrophenol, there was a disappearance of alcohol from the solution, which was approximately equal to the increase shown in the above table when insulin was added to the rat liver flasks. The results are seen in Table II.

Thus, commercial insulin under these conditions is capable of causing the disappearance of alcohol. That this is an oxidative process was shown by displacing the air with nitrogen rather than oxygen, in which case no alcohol was destroyed.

Since commercial insulin is, after all, a tissue extract, and tissue extracts have long been known to be effective in causing the oxidation of alcohol (13, 14), we repeated the experiment using an insulin free pancreatic extract (15). Here again some of the alcohol disappeared from solution, as can be seen from Table II.

In the investigation of insulin in vivo, two human subjects were given a test dose of 1.5 cc. of alcohol per kilogram by vein, and after an interval of an hour blood alcohol determinations were run hourly for three hours, at which time 1 unit

of insulin per kilogram was administered hypodermically, the simultaneous administration of 200 grams of glucose in a small amount of water being effected by mouth. Hourly blood samples were analyzed for alcohol subsequent to the injection. At no time did the subjects evidence any of the signs of hypoglycemic shock. Figure 1 shows the acceleration of the decline of the alcohol curve following the administration of insulin in one of the subjects, amounting to a 50 per cent increase in the rate. The increase in rate of metabolism in each case is seen in Table I.

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